PB-216 558

PROCEEDINGS, SHELLFISH SANITATION WORKSHOP, AUGUST 26-27, 1958

Eugene T. Jensen

Public Health Service
Washington, D. C.

1958

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

- PROCEEDINGS -

1958

SHELLFISH SANITATION WORKSHOP

AUGUST 26 - 27, 1958

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

PB 216 558
Environmental Protection Agency
5555 Ridge Ave., Cincinnati, O. 45213

PROCEEDINGS

SHELLFISH SANITATION WORKSHOP

August 26-27, 1958

Edited by
Eugene T. Jensen
Sr. San. Engr., PHS

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service

Bureau of State Services

Division of Sanitary Engineering Services — Milk and Food Program

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

CONTENTS

	Page
Foreword	1
Agenda	2
Discussions	
(Tuesday Morning)	
Bacteriological Standards for Shucked Eastern Oysters	3
(Tuesday Afternoon)	
Revision of Part I of Shellfish Sanitation Manual	6
(Wednesday Morning)	
Bacteriological Standards for Shucked Eastern Oysters	10
Standards of Identity for Shellfish	11
(Wednesday Afternoon)	
Administration of the Certification Program	12
Construction Criteria for Shellfish Equipment	12
Paralytic Shellfish Poison	13
Disposal of Radioactive Materials as Related to Fisheries	14
Sanitary Significance of Pink Discoloration of Shellfish	14
Conducting Actions	15
Appendices	
Appendix A: Results of a Cooperative Study to Evaluate Present Bacteriological Criteria of Market Oysters (Mr. C. B. Kelly, Chief, Shellfish Sanitation Laboratory, Public Health Service)	16
Appendix B: Results of Bacteriological Analysis of Imported U. S. Shucked Oysters, 1957-1958 (Dr. F. S. Thatcher, Food and Drug Directorate, Canada)	33
Appendix C: Letter from the Chicago Board of Health and Supporting Laboratory Data	37
Appendix D: Bacteriological Quality of Market Oysters in Louisiana (Dr. George H. Hauser, Director, Division of Laboratories, Louisiana State Department of Health)	52
Appendix E: F.D.A. Standards of Identity for Shellfish (Mr. Shelbey T. Grey, Director, Bureau of Program Planning and Appraisal, Food and Drug Administration)	54
Appendix F: Administration of Program Certification of Shellfish Shippers--The Industry Viewpoint (Mr. David H. Wallace, Director, Oyster Institute of North America)	56
Appendix G: Paralytic Shellfish Survey, Maine - 1958 (Mr. Phillip L. Goggins, Marine Resource Scientist, Department of Sea and Shore Fisheries, Maine)	59
Appendix H: A Review of the Paralytic Shellfish Poison Problem in Canada (Mr. Ronald H. Bond, Officer in Charge, Fish Inspection Laboratory, Department of Fisheries, Canada)	64
Appendix I: Washington State Paralytic Shellfish Poison Surveillance (Mr. John G. Girard, Washington State Department of Health)	67
Appendix J: The Significance of Pink Discoloration in Shellfish (Mr. Donald W. Lear, Chesapeake Biological Laboratory, Maryland Department of Research and Education, Solomons, Maryland)	68
Appendix K: List of Persons Attending Workshop	71

FOREWORD

The 1958 Shellfish Sanitation Workshop was arranged by the Public Health Service in accord with the recommendations of the 1956 Workshop. The objectives of the Workshop were: (1) complete the final revision of Part I of the Manual of Recommended Practice for Sanitary Control of the Shellfish Industry; (2) review the progress which has been made in a two-year cooperative study of a bacteriological standard for shucked Eastern oysters at the market level; and (3) discuss other public health problems related to control of the shellfish industry and including topics such as equipment construction criteria, paralytic shellfish poison, and marine disposal of radioactive materials.

Invitations to participate in the Workshop were extended to all State and Territorial Health Departments, Marine Fishery Departments in the coastal States, Health Departments in major cities; interested Federal agencies; Government of Canada; and the shellfish industry trade organizations. The Workshop was attended by ninety-three persons representing forty-three agencies or organizations.

The following proceedings are based on the prepared papers and a partial transcript. The proceedings have been reviewed for accuracy by the various speakers.

Our appreciation is extended to the many persons whose efforts contributed to the success of this undertaking.

Eugene T. Jensen, Chief
Shellfish Sanitation Section
Milk and Food Program
Division of Sanitary Engineering Services

LIBRARY
SANITARY ENGINEERING CENTER
CINCINNATI, OHIO

AGENDA

Tuesday morning, August 26, 1958

CHAIRMAN: Mr. Eugene C. Meredith; Virginia State Department of Health

9:30 Opening Remarks

9:45 A general discussion of bacteriological standards for shucked Eastern oysters

Report on the Cooperative Study of Bacteriological Quality of Oysters Shipped from Chesapeake Bay to Market Areas in New York; Mr. C. B. Kelly, Public Health Service

Canadian Experience with a Bacteriological Standard for Market Oysters; Dr. F. S. Thatcher, Canadian Food and Drug Directorate

Chicago Board of Health Experience with Bacteriological Quality in Shellfish

Bacteriological Quality of Market Oysters in Louisiana; Dr. G. H. Hauser, Acting Director of Food and Drugs, Louisiana Department of Health

Tuesday afternoon, August 26, 1958

CHAIRMAN: Mr. Eugene T. Jensen, Chief, Shellfish Sanitation Section, Public Health Service

1:15 A general discussion of changes in Part I of the revised shellfish sanitation manual including a final decision on each change to be made

Wednesday morning, August 27, 1958

CHAIRMAN: Mr. John D. Faulkner, Chief, Milk and Food Program, Public Health Service

9:30 A discussion of problems in the administration of the cooperative program for the certification of interstate shellfish shippers, and development of action recommendations

The Public Health Service Viewpoint; Mr. Eugene T. Jensen, Public Health Service

The Industry Viewpoint; Mr. David H. Wallace, Director, Oyster Institute of North America

State Viewpoints

Construction Standards for Equipment Used by the Shellfish Industry; Mr. Edwin Ruppert, Milk and Food Program, Public Health Service

Food and Drug Administration Standards of Identity for Shellfish; Mr. Shelbey Grey, Director, Bureau of Program Planning Appraisal, Food and Drug Administration

Wednesday afternoon, August 27, 1958

CHAIRMAN: Mr. David H. Wallace, Director, Oyster Institute of North America

1:45 Panel discussion of paralytic shellfish poison problem in the United States and Canada; Mr. R. H. Bond, Canadian Fishery Inspection Laboratory; Mr. Phillip Goggins, Maine Department of Sea and Shore Fisheries; and Mr. John G. Girard, Washington State Department of Health

2:45 Disposal of Radioactive Materials and its Relation to Fisheries; Dr. Jospeh Lieberman, Chief, Division of Reactor Development, Atomic Energy Commission

4:00 The Significance of Pink Discoloration in Shellfish; Mr. D. W. Lear, Chesapeake Biological Laboratory

5:00 Summary; Mr. John D. Faulkner, Public Health Service

Tuesday morning - August 26, 1958

Mr. Eugene C. Meredith, Chairman

The Workshop convened in the Surgeon General's conference room in the Department of Health, Education, and Welfare building, Washington, D. C. at 9:30 A. M. The Chairman, Mr. Eugene C. Meredith, Chief, Division of Engineering, Virginia State Department of Health, opened the meeting by introducing Dr. T. J. Bauer, Deputy Chief, Bureau of State Services, Public Health Service.

Dr. Bauer welcomed the group on behalf of the Surgeon General, Public Health Service, Department of Health, Education, and Welfare. He pointed out that the shellfish sanitation program is one of the older sanitation activities of the Service and that it was one of the first programs to utilize a cooperative arrangement between Federal, State and local governments and industry to insure safe foods.

He then pointed out that when the program was initiated some 30 years ago the primary interest was in sewage pollution of shellfish growing waters, but that like other programs changes have been required to keep abreast of growth problems. As examples, he cited the emphasis which had been placed on paralytic shellfish poison control within the last five years, and on the present consideration of the problem of radioactivity uptake by shellfish. He also reviewed the accomplishments of the Public Health Service shellfish sanitation laboratory at Pensacola, Florida, and announced that arrangements had been concluded for the transfer of this laboratory to Purdy, Washington to conduct further research on the bacteriology of shellfish.

Bacteriological Standards for Shucked Eastern Oysters:

The Chairman reviewed the development of the interim bacteriological standards for shucked market oysters as recommended by the 1956 Shellfish Sanitation Workshop. He pointed out that in accord with the recommendations of the 1956 Workshop, representatives of the Canadian Department of National Health and Welfare, Public Health Service, New York Department of Conservation, and Departments of Health of New Jersey, Maryland, Virginia and New York City had undertaken a cooperative study of these standards; that representatives of these agencies had met on two occasions to evaluate the study data; and that at the last meeting of this group on June 26, 1958, it was agreed that the results of the study should be presented to the 1958 Workshop by Mr. C. B. Kelly, Chief, PHS Shellfish Sanitation Laboratory.

Mr. Kelly then presented his paper entitled "Results of a Cooperative Study to Evaluate Present Bacteriological Criteria of Market Oysters". His complete paper is included as Appendix A of these Proceedings.

The Chairman asked Dr. Eugene K. Harris, Analytical Statistician, Sanitary Engineering Center, to discuss the statistical significance of the study. Dr. Harris expressed his opinion that the study was statistically sound, but that it was his impression that the false positive percentages were not actually as high as shown and that the false negative percentages were perhaps somewhat higher than indicated. He stated that additional experience with the test procedure would be necessary to confirm this impression.

Dr. F. S. Thatcher, Food and Drug Directorate, Ottawa, Canada, then reported on the Canadian experience with the interim standard. His report is included as Appendix B of these Proceedings.

Mr. Kelly read a letter from the Chicago Board of Health summarizing their experience with the bacteriological examination of shellfish during the 1957-58 season. The letter and supporting laboratory data are included as Appendix C of these Proceedings.

Mr. J. Paul Bowers, Region VII, Public Health Service, presented a paper for Dr. George A. Hauser, Director, Division of Laboratories, Louisiana State Department of Health entitled "Bacteriological Quality of Market Oysters in Louisiana." Dr. Hauser's paper is included as Appendix D of these Proceedings.

The Chairman then opened the meeting for discussion.

Mr. Robert N. Brown, Maryland State Department of Health, moved that the following study group findings be adopted as an interim bacteriological standard for shucked Eastern oysters at the market level:

	"E. coli" per 100 ml.	Standard Plate Count per ml.
Acceptable	≤45	≤50,000
Acceptable-on-condition	>45 ≤230	>50,000 ≤500,000
Rejectable	>230	>500,000

The motion was seconded by Mr. Gregory, Virginia Department of Health.

Mr. Brinsfield, Maryland State Department of Health, inquired if New York City could use the interim standard. Mr. Fortgang, New York City Department of Health, replied that the City might use it on an administrative basis. He explained that the New York City sanitary code is being revised and that the interim standard would very probably be incorporated when it is made a permanent standard.

Mr. Savage, Savage and Mears Oyster Co., asked if the interim standard would be more or less liberal than the present standard. Messrs. Brinsfield and Meredith both indicated it was their opinions that the new interim standard was more realistic, and that industry should have no difficulty in meeting the limits.

Dr. Thatcher compared the proposed standards with the results obtained in Canada during the past year, pointing out that the new standard would have rejected the same shipments as were rejected by the old standard.

Mr. Udall inquired if it was Mr. Brown's intention that the interim standard should be subject to further study during the coming year. Mr. Brown replied in the affirmative.

Mr. Kelly pointed out that the June 26th study group had not made a positive recommendation as to how the fecal coliform organisms would be determined. He suggested that in view of the statistical study made by Sanitary Engineering Center since the June 26 meeting, that fecal coliforms be determined by the E. C. presumptive test. He further stated that in his opinion the E. C. completed test, i.e., IMVIC, is impractical because of time and material demands. He suggested that Dr. Harris discuss the proposed interim standard limits.

Mr. Brown stated that in his opinion "E. coli" was more meaningful than coliforms as indicators of pollution, and from a control standpoint would favor their use. He suggested laboratory people comment on the most desirable methods.

Dr. Thatcher endorsed Mr. Kelly's observations concerning coliforms vs. fecal coliforms as indicators of pollution in shucked oysters and on the use of the E. C. presumptive vs. E. C. completed tests.

Dr. Perry, Maryland Department of Health, endorsed both Mr. Kelly's and Dr. Thatcher's statements pointing out that the E. C. presumptive test might be completed in 24 hours whereas the IMVIC test might require 9 days. He also pointed out that the E. C. test is not specific for E. coli, but that in his opinion all E. C. positive organisms are of fecal origin although they do not necessarily fall into the IMVIC ++-- grouping.

Dr. Harris expressed his opinion that the proposed standards were too stringent, pointing out that the standard would be applied to an individual sample rather than a series of samples, and that a presumptive rather than completed test was to be used. He suggested that a relatively liberal standard be prescribed and recommended that on the basis of statistical analysis the Acceptable value for fecal coliforms be increased from 45 to 130 and the Rejectable limit increased from 230 to 790.

Mr. Wallace emphasized the fact that these "standards" determined, in the mind of the buyer, what is a "good" product. Oysters classed Acceptable-on-condition are seemingly of questionable quality to the buyer and the industry would therefore like to avoid having their product in this category whenever possible. He suggested that Dr. Harris' reasoning also be applied to the Standard Plate Count by increasing the Acceptable limit to 100,000. He suggested Mr. Brown's motion be so amended.

Mr. Pringle pointed out that while an "E. coli" standard might be set on the basis of currently available information it would ultimately be necessary to study the growing areas to determine the original "E. coli" levels.

Dr. Slocum, Food and Drug Administration, stated that it seemed to him that we were now almost at the point of having a useful bacteriological tool. However, he questioned the existence of data relating to the proposed limits to growing area pollution stating that this is a fundamental consideration in the development of a market standard.

Mr. Udell, New York State Department of Conservation, agreed that the market standard and original bacteriological quality of the shellfish were always related. He pointed out further that the most pressing need has been for a market standard and that the cooperative study had been undertaken to fill this need. This should not be construed as indicative of a lack of need for similar information on bacteriological relationships in growing areas.

Mr. Fortgang suggested that a study be made of the relationship between the interim standard limits and observed sanitary conditions in growing areas and plants. In support of this suggestion he quoted a statement made by Dr. Buchbinder as follows: "If a battery of tests such as those we have carried out were performed in conjunction with a simultaneous sanitary survey, some more definite conclusions might be obtained. If presence of organisms of presumed fecal origin is considered as a guide to oyster control and laboratory findings could be correlated with a simultaneously performed sanitary survey, then facts would be available with which to look at the problem from a fresh point of view." Mr. Fortgang's suggestion was then rephrased as a motion, seconded, and carried.

Mr. Brinsfield indicated Maryland had considerable information on the presence of E. C. positive organisms in oysters as harvested which indicated an initial low concentration with some increase during handling.

Dr. Novak, Louisiana State University, outlined some of his experimental work at the University and stated emphatically that a 50,000 Standard Plate Count "Acceptable" limit would place a substantial portion of Louisiana oyster production in the "Acceptable-on-Condition" category. He urged very strongly that Mr. Wallace's suggestion for increasing this limit to 100,000 be adopted.

Mr. Woodfield, Woodfield Fish and Oyster Company, stated that it was apparent that the control officials were not in agreement on the limits to be adopted and urged that a liberal standard be accepted in fairness to the industry.

Dr. Thatcher indicated his Department could probably accept the 100,000 Standard Plate Count limit for the Acceptable class.

Mr. McGinnes, Virginia Seafoods, Inc., inquired at what bacterial level the shellfish might become dangerous.

Mr. Kelly replied that such limits were very difficult to arrive at by epidemiological study and that it was the responsibility of the control officials to insure that the product was absolutely safe, rather than to see how close they could come to an unsafe product.

Mr. McGinnes agreed with this, but stated that he did not think a needlessly restrictive standard should be imposed unless it was actually indicative of a hazard.

Mr. Udell proposed that the standard suggested by Mr. Brown be tested for a reasonable length of time and that the control agencies be liberal in the interpretation of results. He pointed out, however, that fecal coliforms are indicative of direct fecal contamination and are of much greater significance than coliforms.

Dr. Novak again reiterated his stand for an "Acceptable" Standard Plate Count limit which would not discriminate against the Louisiana oyster producers.

Mr. Kelly stated that his experience with these oysters agreed with that of Dr. Novak. He further stated that in his opinion the proposed E. C. limit of 45 did not give sufficient recognition to the statistical variations which should be expected. He urged that the limits suggested by Dr. Harris be substituted.

Mr. Brown requested a vote on the original motion. The vote was taken and the motion defeated. Mr. Wallace then proposed that Mr. Brown's proposal be restated to incorporate Drs. Harris' and Novak's suggestions with the understanding that the interim standard was to be used and evaluated for a two year period:

	"E. coli" per 100 ml.	Standard Plate Count per ml.
Acceptable	≤130	≤100,000
Acceptable-on-condition	>130 ≤790	>100,000 ≤ 500,000
Rejectable	>790	>500,000

Dr. Perry pointed out that the E. C. positive results represented bacteria of fecal origin which have significant public-health importance. He suggested that the 790 value was much too high, and urged that values of 45 and 230 be substituted for the E. C. limits.

Dr. Harris replied that there was no significant difference between an MPN of 130 and 230 since this is a difference of only one positive tube.

Mr. Kelly agreed with Dr. Harris, pointing out that the 130 - 790 limits were justified on the basis of probability. Mr. Wallace said that the industry wanted a safe standard and suggested that the 790 be changed to 490 to at least partially meet Dr. Perry's objection.

Mr. Brown pointed out that industry had not been hurt by the 1956 interim standard which was quite liberal and which was administered liberally by the receiving States and Canada. He suggested conservatism in the establishment and administration of a standard which would be subject to reevaluation and possible revision in two years. The motion, as above stated, was then seconded and carried by a show of hands. There were a number of dissenting votes.*

Tuesday afternoon - August 26, 1958

Mr. Eugene T. Jensen, Chairman

Mr. Jensen stated that the objective of the afternoon session should be to complete the revision of Part I of the shellfish manual. He explained that the first review draft was sent to the coastal States, U. S. and Canadian Federal Agencies and the oyster industry in January 1958, and that a second review draft was circulated to all interested States and Federal agencies, and the oyster industry for review in May 1958. Public Health Service headquarters and regional representatives then discussed the second review draft with State agencies and the shellfish industry in a series of meetings in Vancouver, B. C., New Orleans, Boston, New York, Baltimore, Washington, Richmond, and Raleigh. To assist the Workshop in making this final review, the suggested changes in the second review draft had been tabulated and referenced to the review draft. Each such change was then discussed individually with the following results:**

1. Format: It was decided the manual would be printed on 8" x 10½" paper (the same as Part II) and that each page would be dated and punched to facilitate future revision.

2. Effective Date: It was decided that except where otherwise indicated the manual would become effective 60 days after publication.

3. Coliform Definition: It was decided that the definition of "coliform" should also refer to the E. C. positive organisms. Dr. Novak, Louisiana State University, suggested amending the coliform definition as follows: "Bacteria of this group (coliforms) which will produce gas from E. C. Medium within 48 hours at 44.5°C. in a water bath will be referred to as 'fecal coliforms'". This suggestion was accepted by the Workshop. It was decided that the definition should not refer to air incubation temperatures because the method has not yet been accepted by the APHA Standard Method Committee.

4. Shellfish Definition: It was agreed that the definition should include the recommendations of the 1954 National Conference on Shellfish Sanitation as follows: "Shellfish products which contain any material other than the meats and/or shell liquor of oysters, clams, or mussels, will be regarded as a processed food and will not be included in the Cooperative Program."

---

* The discussion of the interim standard was reopened on Wednesday morning.

** Minor changes in wording which were accepted by the Workshop without discussion are not reported.

5. Sewage Discharges from Boats: The need for a Cooperative Program requirement for legal authority to regulate the discharge of sewage, radioactive; and other toxic wastes from boats in the vicinity of approved shellfish areas was considered. It was agreed that such a requirement was desirable, but infeasible at present. Mr. Udell (New York State Department of Conservation) indicated his organization is now studying this problem.

6. Identification of Shellfish: The new requirement prohibiting Cooperative Program shippers from possessing or selling shellfish from out-of-State sources that had not been produced in accord with Program requirements was discussed at some length, particularly certification of offshore sea clam beds which are outside the jurisdiction of a State. Mr. Jensen stated that this latter problem was of such magnitude that it could not be solved by the Workshop and suggested that no action be taken.

Mr. Wallace pointed out that the adoption of this requirement would prohibit State certified dealers from handling shellfish produced in any foreign country other than Canada. His statement was not disputed. The changed requirement was accepted by the Workshop.

7. Minimum Plant Sanitation Requirement: Mr. Jensen read the following telegram from the Pacific Coast Oyster Growers Association: "Pacific Coast Oyster Growers Association in convention assembled in Olympia, August 23, instructed that you be advised of the concern of this Association of the Public Health Service becoming involved in the cooperative shellfish program in any manner contrary to its historical responsibilities. Public Health Service should continue to either endorse or refuse to endorse producer States programs relating to shellfish sanitation in a manner that takes into consideration the responsibilities of the State shellfish regulatory authority. /S/ Pacific Coast Oyster Growers Association, Chas. R. Pollock, Secretary."

There was an extensive discussion of the manual provision requiring that all certified shellfish shippers meet the Part II construction requirements at the time of certification, and that they maintain a sanitation rating of at least 80% during periods of operation. The relationship of this requirement to the PHS plant inspection report, PHS-769, was also discussed. It was agreed that only the State had the right to issue, suspend, revoke or reissue shipper certificates, and that the PHS should not interfere with this process. It was pointed out that the approval of growing areas has much greater public-health significance than certification of plants, and that the former is accepted as a State function. It was agreed that establishment of the plant certification criteria was justified, and that they should become effective September 1, 1959. It was also agreed that plant ratings should preferably be determined on the basis of a PHS inspection report form, but that inspections reported on State forms comparable to PHS-769 should also be accepted.

8. Monthly Summary of State Inspection Activities: There was considerable discussion of the manual requirement that States submit a monthly summary of inspection and patrol activities to the PHS Regional Office. It was the consensus that such reports were necessary. It was agreed that PHS should provide the necessary summary forms.

9. Frequency of Inspection of Certified Shippers: The draft manual stipulated that shucker-packers and repackers be inspected monthly and that shell stock shippers be inspected at least twice yearly. The inspection frequency for shucker-packers and repackers was acceptable to the Workshop; however, there was an extensive discussion of the inspection frequency for shell stock shippers and reshippers.

Mr. Udell, New York Conservation Department, suggested that this was a regional problem and might best be solved on that basis. This proposal was acceptable to the Workshop and it was agreed that the PHS would arrange a series of regional meetings with the States to discuss the entire problem of certification and inspection of shell stock shippers and reshippers.

It was further agreed that this manual item regarding the inspection of shell stock shippers and reshippers would be rewritten as: " . . . necessary to provide adequate public-health supervision of their operations".

10. Inclusion of Intrastate Shippers on PHS Shellfish Shippers List: It was agreed that intrastate shippers might be listed if States so desired. Mr. Jensen stated the Service was opposed to the listing of any appreciable number of such shippers because of the cost of producing and distributing the PHS list of certified shippers.

11. Intrastate Sale of Market Shellfish: The draft of the manual proposed that all shellfish shippers meet Cooperative Program sanitary standards even though they sold only in intrastate commerce. Several States had objected strongly to this requirement.

It was agreed that it would be desirable to apply equivalent sanitary standards to both intra- and interstate shippers, but that this was not feasible under the present administration framework of the Cooperative Program. Mr. Jensen suggested that the manual item be rewritten to recommend rather than require application of these standards to intrastate shippers. He stated that any evidence that intrastate dealers were shipping interstate or were selling to interstate shippers would be considered as a defect in the State's administration of the Cooperative Program, and that the Service would expect the States to show that such dealers operate only intrastate. He suggested that the Workshop go on record as strongly recommending the use of substantially equivalent standards for both classes of shippers.

Mr. Wallace stated that industry also opposed dual standards because (1) they tend to place the interstate dealer at a competitive disadvantage in the local markets, and (2) the interstate certification system might be damaged if there was a disease outbreak attributable to a product from such a shipper.

The Workshop decided to accept dual standards insofar as the manual was concerned by adopting a resolution strongly recommending the use of substantially equivalent sanitary standards for intra- and interstate shellfish shippers.

12. Growing Area Designations: It had been recommended that the areas designations for "moderately polluted-restricted" be changed to "RESTRICTED" and "grossly polluted-closed" to "PROHIBITED", to recognize the fact that areas might be closed for reasons other than sewage pollution, and to make the designations easier to use. There was a brief discussion of this proposal and its relationship to existing State laws. The Workshop agreed that the change should be made.

13. Paralytic Shellfish Poison: The draft manual specified that States should close growing areas whenever the poison content of the shellfish reaches a level of 80 micrograms per 100 grams of meats (400 mouse units assuming a conversion factor value of 0.2). Mr. Jensen explained that this quantitative level was based on Canadian experience, particularly in the Maritime Provinces. He stated that the 80 micrograms level was not, in itself, dangerous but that it was indicative of a situation in which a very rapid rise might be expected. Mr. Bond pointed out that a serious case of shellfish poisoning in a small child had been caused by about 100 micrograms and that the death of an adult had been reported at Metis Beach, Province of Quebec, due to an estimated 400 micrograms. He indicated that the Canadian control limit of 400 mouse units (64 micrograms) was completely acceptable from their standpoint and that they intended to continue to use that value as the control level. He cautioned against adoption of more liberal standards.

Mr. Girard objected to the 80 micrograms control level pointing out that different species of shellfish were involved in the State of Washington. He stated that in some instances butter clams (Saxidomus) may contain some toxin year-around. He strongly suggested that a slightly higher control level, perhaps 120 micrograms, be adopted to enable them to keep these beds open. He based his suggestion on the assumption that a low level of toxicity was normal in some species of clams in this State, and that there had been no illness attributable to the clams. Since shellfish poisoning is quite spectacular he assumed that the State health department would have heard of any outbreaks. Mr. Bond replied that the State might not necessarily have heard of such outbreaks citing his own experience in the Maritime Provinces in which they had learned of poisoning cases only by accident.

Mr. Wallace stated he had discussed this problem with the Pacific Coast Oyster Growers Association at their recent convention and that PCOGA also opposed the 80 micrograms control level. The industry based their opposition on the same grounds as outlined by Mr. Girard.

Dr. D'Aguanno, Food and Drug Administration, described the FDA toxicity standards as follows: For whole clams, fresh, frozen or canned, the average toxicity shall not exceed 400 mouse units per 100 grams and no individual package shall exceed 2,000 mouse units. With minced clams, the average toxicity must be less than 2,000 mouse units per 100 grams and no individual package shall assay materially above 2,000 mouse units. He pointed out that these standards, adopted about 10 years ago, were based on studies made by the Food and Drug Administration as reported at the Public Health Service's 1955 shellfish toxicology conference. He expressed doubt that the Administration would favor any liberalization of this standard in view of experience indicating the toxin is more powerful than was realized a few years ago.

Mr. Odell suggested the possibility of an interim standard pending development of additional data. Mr. Bond pointed out that they had sufficient experience in Canada to justify their present standard. Dr. D'Aguanno again pointed out that there was also an existing FDA standard.

Mr. Jensen suggested that in view of the Canadian experience and existing FDA standards that the suggested tolerance of 80 micrograms be adopted by the Workshop with full recognition of the fact that this standard was based largely on experience with the poison in the Maritime Provinces of Canada; that the justification for applying this standard on the West Coast was doubtful; and that there was a need for further research on the problem. The Workshop agreed to this suggestion and the 80 microgram control limit was accepted. It was agreed that the discussion on research needs would be deferred until after the Wednesday afternoon discussion of shellfish poison.*

14. Minimum Relaying Time: The review draft of the manual proposed a relaying time of 4 to 14 days depending upon the initial sanitary quality of the shellfish; however, most of the States favored a minimum of 14 days. Mr. Udell pointed out that it might be better to require only that relaid shellfish be kept in the clean area for a period of time sufficient to allow cleansing. He pointed out that there were many variables involved which made it difficult to define a minimum relaying period for all species of shellfish under all conditions. This suggestion was acceptable to the Workshop.

15. Laboratory Control of Controlled Purification: Dr. Thatcher suggested that under routine operating conditions the before-purification bacteriological examination of each lot of shellfish would not contribute significantly to public-health control of the operation, but that it would increase operating costs.

It was agreed that this examination could be omitted if all shellfish were obtained from the same area and were of comparable bacteriological quality. However, it was also agreed that before-and-after bacteriological examinations would be necessary if shellfish from varying geographic localities or of varying bacteriological quality were to be purified.

16. Public Health Service Plant Inspection Report: There was a brief discussion of the inspection report and the values assigned to each item. Mr. Jensen explained that the inspection report had been developed in cooperation with the Maryland State Department of Health, and that the item values were assigned empirically so that a well-constructed, well-operated plant would attain a score of at least 95%, and a poorly-operated, poorly-constructed plant would have a score of less than 80%. He explained that the inspection report had been used for the past year by the PHS shellfish sanitation consultants with satisfactory results in all parts of the country. The PHS Regional shellfish sanitation consultants, Messrs. Macomber, Fletcher, Carley, Bowers, and Houser, confirmed that they had used the inspection reports in evaluation work and that it appeared to give a fair representation of plant conditions. It was brought out in the discussions that while most plants rated over the limiting 80% value, several plants rating in the 50% to 60% range had been found, and that one certified establishment had rated o. ' 11%.

Mr. Jensen pointed out that these results were in contrast to results obtained on the old PHS inspection report which almost invariably gave scores exceeding 90% regardless of the adequacy of construction or operation.

He then explained that there had been some controversy over interpretation of the water supply item due to difficulties in interpreting the requirements of PHS Publication No. 24, "Individual Water Supply Systems," which forms the basis for this item.

Several State representatives also stated that it was difficult to interpret this item particularly in the case of the older wells. They asked if bacteriological sampling could be accepted in lieu of positive information on the type of well construction. It was agreed that bacteriological sampling was important, but that it was not a complete substitute for a sanitary evaluation of the well. Mr. Jensen cited two instances of wells which were not properly constructed, but which gave acceptable bacteriological results.

It was proposed that the item might be subdivided so that only part of the 15% would be deducted if the well did not meet all construction requirements of PHS Publication No. 24. It was pointed out by a State representative that a well was safe or it wasn't safe, and that he opposed any system of partial credit for well construction.

Mr. Faulkner pointed out that the problems faced in shellfish sanitation were not unlike those in the dairy industry and cited the PHS Milk Ordinance and Code water supply requirements, also based on PHS Publication No. 24, as an example of how the problem might be handled. The Milk Ordinance and Code has specific construction requirements; however, exemptions are made for wells which have already been constructed.

---

* The remainder of the shellfish manual items were discussed on Wednesday morning, but are included herewith as a part of the Proceedings.

After extensive discussion it was agreed that each well would have to be considered on its own merits. The PHS shellfish sanitation consultants will, therefore, evaluate each well with their State counterparts and will reach a decision on the adequacy of its construction, and if it is decided jointly that the well is safe, the full 15 points will be credited.

The Chairman, Mr. Wallace, stated that he believed it would be appropriate for the Workshop to go on record as adopting the manual, as revised, as the new guide for the shellfish certification program. A motion to this effect was made, seconded and passed without dissenting votes.

Wednesday morning - August 27, 1958

Mr. John Faulkner, Chairman

In opening the discussion the Chairman pointed out that there had been considerable private discussion of the interim bacteriological standard adopted Tuesday, and that there was some dissatisfaction with the action which had been reached. He suggested the topic be reopened for discussion.

Mr. Udell stated that it was his opinion that the Workshop had voted in haste on the interim standard and that he agreed there should be further discussion. He proceeded to outline the development of the coliform standards in shellfish sanitation, pointing out that the coliform MPN system was originally used to evaluate the effectiveness of water treatment plants; that the system was then applied to shellfish growing areas, and finally to the shellfish. He pointed out that there had been many studies of shellfish bacteriology during the past 50 years the most recent of which was the cooperative study of the past two years which had indicated that the E. C. positive coliform organisms were superior to coliforms as a contamination index for shucked oysters.

He stated that there appeared to be some feeling on the part of industry that the regulatory agencies were being too restrictive. He assured industry that these agencies were only trying to find a reasonable yardstick to measure the quality of market oysters so as to protect the rights of the consuming public. He further stated that such a standard must consider the bacteriological quality of the shellfish at three different levels:

1. As harvested and delivered to the packing house. At that point the fecal coliform MPN should be negative or very low, and the Standard Plate Count should also be relatively low.

2. During shucking and packing. Some increases are expected and the yardstick should indicate if the opefations are satisfactory.

3. During marketing. A rapid increase in bacteria counts is indicative of poor refrigeration or mishandling.

Mr. Udell indicated he agreed with Mr. Wallace's statement concerning the acceptable-on-condition category, and that as a regulatory agency he would also like to avoid the category. To recognize the sanitary significance of the E. C. positive coliforms; the normal plate counts in Louisiana oysters; and the statistical variations of the MPN, he proposed the following one-year interim standard:

Acceptable: An E. C. "E. coli" MPN of 78 or less per 100 ml. and a SPC of 50,000 or less per ml. provided, however, that an E. C. MPN of 230 per 100 ml. and a SPC of 100,000 per ml. be accepted in occasional samples with the provision that the producing area control agency would be notified of successive results in the latter range.

Rejectable: An E. C. MPN exceeding 230 per 100 ml. or a SPC exceeding 500,000 would be considered unsatisfactory and subject to rejection by the State regularory authority. The acceptability of future shipments would depend upon an investigation by the State regulatory authority.

Dr. Novak again reiterated his stand as opposing adoption of a Standard Plate Count limit which could not be met by Louisiana oysters. In response to a question from Mr. Udell he replied that Standard Plate Counts in the 70 to 90 thousand range might be expected at any time of the year.

Mr. Kelly also pointed out that such plate counts were characteristic of oysters from the area, and that he believed handling by the harvesters was satisfactory and therefore not

responsible for the counts. He referred to Dr. Hauser's paper on the bacteriology of the Louisiana oysters as evidence of this.

Mr. Udall agreed that this was a valid objection to his proposal and stated he would be willing to change the plate count limit as suggested by Dr. Novak.

Dr. Lewis and Dr. Harris pointed out that there was a gap in the Standard Plate Count limits between 100,000 and 500,000. They suggested that this be reconciled by using a 500,000 limit for the Rejectable category. This suggestion was acceptable to Mr. Udall.

Mr. Brinsfield suggested that the interim standard be adopted for a two-year rather than one-year period to give the States ample time to evaluate the results obtained. This suggestion was also accepted by Mr. Udall.

The standard as originally proposed was then restated as follows:

An Interim Bacteriological Standard for Fresh and Frozen Oyster Meats at the Market Level

Satisfactory*

E. coli density of not more than 78 MPN per 100 ml. of samples as indicated by production of gas in E. C. liquid broth media nor more than 100,000 total bacteria per ml. on agar at 35°C. will be acceptable without question.

An E. coli content of 79 to 230 MPN per 100 ml. of sample or a total bacteria count of 100,000 to 500,000 per ml. will be acceptable in occasional samples. If these concentrations are found in two successive samples from the same packer or repacker, the State regulatory authority at the source will be requested to supply information to the receiving State concerning the status of operation of this packer or repacker.

Unsatisfactory*

E. coli content of more than 230 MPN per 100 ml. of sample or a total bacteria count of more than 500,000 per ml. will constitute an unsatisfactory sample and may be subject to rejection by the State shellfish regulatory authority. Future shipments to receiving markets by the shipper concerned will depend upon satisfactory operational reports by the shellfish regulatory authorities at the point of origin.

Mr. Wallace moved that the Workshop rescind their previous action relative to an interim standard and that the above interim standard be adopted for a two-year period.

Mr. Woodfield inquired as to what action would be taken if an "E. coli" value between 78 and 230 was attained. Mr. Udall replied that no action would be taken on one sample, but that if successive samples showed this level, the control agency in the producing State would be notified.

Mr. Wallace's motion was then seconded and passed without dissenting vote.

The Chairman then raised the question of how the interim standard evaluation study would be carried out. It was agreed that Mr. Kelly would develop an evaluation procedure which he would submit to the States. It was further agreed that representatives of the interested agencies would meet at Virginia Beach, Virginia, during the week of September 15, 1958 at the Chesapeake Bay Seafood Seminar to work out the details of the study.

Mr. Berry, Virginia Department of Health then announced that the Chesapeake Bay Seafood Seminar being held at Virginia Beach, Virginia, was an open meeting, and invited all interested agencies to have representatives attend.

A paper entitled "Standard of Identity for Shellfish" was presented by Mr. Shelbey Grey, Director, Bureau of Program Planning and Appraisal, Food and Drug Administration. Mr. Grey's paper is included as Appendix E of these Proceedings.

---

* See page 6, item 3, for a definition of the term "E. coli" as used in the interim standard.

Wednesday afternoon - August 27, 1958

Mr. David Wallace, Chairman

Administration of the Cooperative Program for the Certification of Interstate Shellfish Shippers:

Mr. Jensen discussed the Public Health Service responsibilities in the Program pointing out that the Service recognizes the difficulties in communications between the interested groups and the problem of obtaining uniform interpretations of requirements. He suggested the following corrective actions:

1. Establishment of seafood seminars in each producing region similar to those now organized on the West and Gulf Coasts and the Chesapeake Bay area, and that provisions be made for the exchange of information between these seminars.

2. Organization of Shellfish Sanitation Workshops at two or three year intervals.

3. Development of a PHS manual detailing the procedures to be followed in the review and evaluation of State shellfish sanitation programs. He indicated that the Service planned to develop such a guide, and that review drafts would be circulated to all interested State and Federal agencies and the shellfish industry.

Mr. Wallace then discussed the industry views of the certification system emphasizing that they were particularly concerned about lack of uniformity in interpretation of requirements, and by the acceptance of low sanitary standards for small intrastate shippers. His prepared paper is included as Appendix F of these Proceedings.

Construction Standards for Equipment Used by the Shellfish Industry:

Mr. Ruppert, Public Health Service, explained that both the shellfish industry and equipment suppliers had expressed a need for a guide to the construction of equipment used by the shellfish industry. He pointed out that Item 14, Part II of the shellfish manual gives the general requirements for such equipment but that it is not an adequate guide for the construction of purchase of equipment. Consequently, a substantial portion of the equipment used by industry is substandard in fabrication although made up of satisfactory materials. He further pointed out that many of these problems were regional in nature citing the example of the returnable shipping container used in the Chesapeake Bay area but not on the West or Gulf coasts.

Mr. Hobbs indicated that it might be possible to review equipment at the Chesapeake Seafood Seminar, and mentioned that several equipment suppliers had been invited to have samples of their equipment available at that meeting.

Mr. Udell stated that for many years there had been difficulties with equipment, citing as examples requirements for cleanable blower lines and drain valves, and the need for equipment for precooling shucked shellfish. He suggested that an official approval system would be very helpful.

Mr. Ruppert replied that it would be difficult for the Public Health Service to approve equipment, but that he thought the Service could act as a clearing house for such information from the various States. He also indicated that the Service could develop general construction criteria which would be useful to equipment fabricators.

Mr. Wallace viewed this as a matter of considerable importance. He pointed out that many individual packers had attempted to write their own specifications for equipment and that this had resulted in use of expensive, custom-built equipment which could have been avoided if there had been some method of obtaining health department "approval" of equipment. He strongly urged that the regulatory agencies develop such standards. He pointed out that such construction standards would also be helpful to the equipment suppliers in fabricating items which would be acceptable to all State control agencies.

Mr. Jensen again stated that it was not feasible for the Public Health Service to approve specific equipment items, but that the Service could develop construction criteria for equipment. He suggested that this might be undertaken with the help of the State agencies and that Regional Seminars might be the best approach to the problem. He cited the example of the review of a paper oyster shipping container by the 1958 Gulf Seafood Seminar as an example of how this procedure might operate.

Mr. Hobbs, Maryland Department of Health, indicated that they had recently been asked for their opinion on the plastic shipping container and presented a sample of the plastic. Mr. Ruppert replied that the Food and Drug Administration had a list of plastics approved for use in the food industry and that FDA opinion should be sought regarding the toxicity of such materials.

Mr. Hobbs moved that the Public Health Service organize a committee to develop plans for standard equipment for use in the shellfish plants. The motion was seconded and adopted without dissenting votes.

Panel Discussions of Paralytic Shellfish Poison Problem in the United States and Canada:

The Chairman introduced the panel members pointing out that the 1957 outbreaks of paralytic shellfish poison on the East and West coasts had shown the importance of this topic. He suggested that discussion be withheld until after each of the panel members had presented their papers.

Mr. Philip Goggins, Maine Department of Sea and Shore Fisheries, presented a paper entitled "Paralytic Shellfish Survey, Maine, 1958." A copy of his paper is included as Appendix G of these Proceedings.

Mr. R. M. Bond, Canadian Fish Inspection Laboratory, presented a paper entitled "A Review of the Paralytic Shellfish Poison Problem in Canada." Mr. Bond's paper is includud as Appendix H of these Proceedings.

Mr. John Girard, Washington State Department of Health, presented an informal discussion of paralytic shellfish poison problems on the West Coast. (See Appendix I.)

The Chairman then indicated that there were several other persons in attendance who had considerable experience with the paralytic shellfish poison and asked that they also give their ideas on this problem.

Dr. Edward J. Schantz, Army Chemical Corps, stated he had been interested in the toxicity problem for many years and that his organization had isolated the poison in pure form from the Alaska butter clams. He also indicated that they had never identified the original source of the poison in Alaska and that, in his opinion, some knowledge of this was essential. He also pointed out that the butter clam accumulates the poison at a slower rate than mussels or soft clams and that it is stored for a much longer period of time, perhaps years.

Mr. Henry Meyers, Arctic Health Research Center, stated that there had only been one fatality in Alaska due to paralytic shellfish poison in recent years. (Public Health Reports, Vol. 70, No.4, April 1955 "Shellfish Poisoning Episode in False Pass, Alaska"). He indicated that they had not been able to find Gonyaulax catenella in the waters at that time but they had since found small numbers in Alaska waters.

Dr. Daniel B. Quayle, Pacific Biological Station, British Columbia, Canada, stated that there were some anomalies in the West Coast situation. As an example he pointed out that different species of shellfish living under the same conditions on the same beach accumulate toxin at different rates. He indicated Gonyaulax catenella had been found in British Columbia waters, and that he recognized this organism was associated with the poison in California. However, he questioned if this was the only possible causative organism in British Columbia waters. He mentioned that in the past, paralytic shellfish poison in British Columbia had been concentrated along the ocean side of the island. In contrast, the 1957 outbreak of paralytic shellfish poison originated in the Straits of Georgia, an enclosed body of water in which shellfish poison had not been previously reported in significant amounts.

Dr. D'Aguanno, Food and Drug Administration, stated that his organization was very interested in the sampling work that the Canadians were doing and stated that he would like to receive monthly reports of this. Mr. Jensen replied that such arrangements had been made and that copies of the Canadian sampling results were routinely forwarded to the FDA Boston office.

Dr. D'Aguanno, FDA, asked what the conversion factor was for the Canadian sampling. Dr. Tennant stated that they had a CF value of 0.16, i.e., 400 m.u. equals 64 micrograms.

Dr. Schantz stated that it was very important that all laboratories use the standardized assay procedure and that they report their results in micrograms rather than mouse units to facilitate comparison of data. As an example of this, he compared the Canadian conversion factor value of 0.16 with the Maine Sea and Shore Fisheries conversion factor value of 0.31, a difference of almost 100% that is eliminated by the use of the reference standard in the assay procedure.

A question was asked as to how the 80 microgram control level was derived. Dr. Lewis, Sanitary Engineering Center, replied that this value was based on the original Canadian quarantine level of 400 m.u. He pointed out that conversion factors of the 11 laboratories which had participated in the study of the bioassay procedure had an average CF value of 0.19. This was rounded off to 0.2 and the 400 m.u. control was restated as 80 micrograms.

Mr. R. E. Watson, Ralph E. Watson Oyster Co., asked if there was shellfish poison in the Southern waters.

Dr. Schantz replied that the poison was frequently found in Washington and California and that Gonyaulax had also been found in the Gulf Coast but that paralytic shellfish poison had never been reported in the latter area.

Mr. Watson remarked that "red tide" was killing fish in Chesapeake Bay and wondered if there was any relationship between this and shellfish poison. Mr. Jensen reported that clams from Chesapeake Bay had been assayed with negative results.

Mr. Girard asked Mr. Bond how many clams were ordinarily collected for samples and if the variation in toxicity within an area had been established? Mr. Bond replied that ordinarily 24 shellfish were picked up for each sample. He then pointed out that rather wide variation in sampling results might be expected in a short distance. He cited the example at their Head Harbour station where the samples were usually collected at a mooring pole. Samples collected from the intertidal zone approximately 200 feet distant were approximately 50% lower.

The Chairman suggested that paralytic shellfish poison was a very real problem both to the industry and control agencies and stated that, in his opinion there was an apparent need for further research on the causative organism and its relationship to the shellfish.

Mr. Goggins moved that the Workshop go on record as urging further research on the causative organism and on the relationships of the organisms to the poison in shellfish. The motion was seconded and carried without dissenting votes.

Disposal of Radioactive Material and its Relation to Fisheries:

Dr. Joseph Lieberman presented an informal paper on the disposal of radioactive material and its relation to fisheries.

Dr. Chipman pointed out that for the past 8 years the Fish and Wildlife Service Radiobiological Laboratory has been investigating the up-take of radioactive materials by shellfish and that he believed this work has shown that shellfish are good accumulators of such material and thereby emphasize the danger of sea or estuary disposal of highly radioactive wastes. He agreed with Dr. Lieberman that the storage of certain types of radioactive wastes is currently feasible but he felt that the problem will become continuously worse and that we are not prepared to meet the waste disposal problems of the next few years. Dr. Lieberman expressed doubt that we would ever be able to dispose of high level materials at sea because of loss of control and pointed out that there were other hopeful methods of disposing of radioactive wastes such as disposal into geological formations, and/or fixation in inert solids. He also noted that he did not believe that problems associated with radioactive waste management would bottleneck the safe development of the atomic energy industry.

Mr. Girard inquired if there was any evidence of shellfish in Willapa Harbor being contaminated by radioactive wastes in the Columbia River. Dr. Lieberman replied that he knew of no proven effect on the shellfish in that area.

Mr. Miles asked what the final conclusion was on the disposal of radioactive wastes near Australian oyster growing areas that had been referred to. Dr. Lieberman said that, as he recalled the study, only small quantities of low level wastes could be disposed of in the area, and that in general they would have to meet the maximum allowable concentrations for drinking water.

Sanitary Significance of Pink Discoloration of Shellfish:

Mr. Donald Lear presented a paper entitled "The Significance of Pink Discoloration in Shellfish," copy of which is included as Appendix # of these Proceedings.

Mr. Goggins stated that they had also noticed pink coloration in shellfish from the Machiasport and Lubec Channel areas, usually early in June. He stated that the coloration, apparently associated with a marine rotifer, was noticeable as soon as the shellfish were shucked.

Mr. Kelly asked Mr. Lear how the clams looked after steaming or boiling. Mr. Lear replied that heat destroys the pigment and that the shellfish could be sold if they were breaded and frozen so that the color was not apparent to the consumer.

Mr. Kelly stated that it seemed that the color was due to food organism and that there was little that could be done about it. He suggested it might be best to sell only shell stock since the color does not ordinarily develop immediately after opening. Mr. Lear replied that consumer resistance would be the determining factor in such sales.

Mr. Hobbs stated that oysters which had been frozen on the decks of oyster boats were sometimes bloody and asked if this was in any way related. Mr. Lear stated that their studies had been with clams and that he would be unable to say if the color in oysters was due to the same organism.

Concluding Actions:

The Chairman asked if the group felt that it would be desirable to schedule another shellfish sanitation workshop. Mr. Hobbs moved that the Public Health Service arrange for another workshop in two years. The motion was seconded and carried without dissenting vote. Mr. Girard moved that the next Workshop be held in Chicago. No action was taken on his motion.

Mr. Pringle stated that he wanted to take the opportunity to commend Mr. Kelly for his work in Pensacola and for the aid he has given the Southern States with their shellfish sanitation programs. The Workshop membership acknowledged this with a standing vote of applause.

Motion for adjournment was then introduced. The motion was seconded and carried and the Workshop adjourned.

APPENDIX A

RESULTS OF A COOPERATIVE STUDY TO EVALUATE PRESENT BACTERIOLOGICAL CRITERIA OF MARKET OYSTERS

Prepared for the Cooperating Agencies
By
C. B. Kelly, Chief
Shellfish Sanitation Laboratory

Robert A. Taft Sanitary Engineering Center
Bureau of State Services
Public Health Service
U. S. Department of Health, Education, and Welfare

Possibly one of the most perplexing problems in sanitary control of shellfish at the present time is the establishment of a safe but reasonable set of bacteriological criteria for shellfish as received at the market. The problem involves the selection of suitable bacterial indices and numerical limits for these organisms that not only will judge the extent of mishandling of the food product during processing and shipping, but at the same time indicate whether or not the shellfish was harvested from areas subject to dangerous human or animal contamination.

The question of bacteriological criteria for market oysters received serious attention at the 1956 Shellfish Sanitation Workshop. Consuming and producing States and the Public Health Service presented a collection of data which put serious doubt on the feasibility of the bacteriological criteria as they appear in the 1946 edition of the Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry. It was developed at that time that even in well designed and well operated shellfish establishments it was not possible in certain areas to produce shellfish whose bacterial content was within the stipulations of the 1946 Manual.

During the past few years increased interest has been seen on the part of shellfish control agencies in the determination of bacteriological quality of shellfish as received at the market. Some agencies established either official or tentative working standards by which they could at least classify the product as received. Possibly the most notable of these is the classification system established by Canada in 1951. This groups the shellfish into three classes, acceptable, acceptable on condition, and rejectable, which allows judgment in whether or not a product should enter or be tolerated in the consuming area. The acceptable on condition classification has the added advantage of encouraging investigation on the part of the producing States of the plants producing a mediocre product, detecting and eliminating somewhat unsatisfactory conditions with the result of affecting an over all improvement in quality of the product. Because of the success of Canada with this scheme, the method was accepted in principle at the Shellfish Sanitation Workshop but modified by increasing the upper limit coliform MPN in the acceptable class from 2,400 to 16,000. The bacteriological criteria established at the Shellfish Sanitation Workshop are as follows:

> Class 1. Acceptable: Shucked oysters with a Most Probable Number (MPN) of coliform bacteria of not more than 16,000 per 100 ml., and/or a Standard Plate Count of not more than 50,000 per ml.
>
> Class 2. Acceptable on Condition: Shucked oysters with a coliform MPN greater than 16,000 per 100 ml. but less than 160,000 per 100 ml., and/or a Standard Plate Count greater than 50,000 per ml. but less than 1,000,000 per ml. (The oysters will be accepted on the condition that the shellfish sanitation authority in the originating State will make immediate investigations of the producer's plant and operations, and will submit a report of such investigations to the control agency in the market area. On the basis of this report the control agency in the market area will reject of permit further shipments from the producer in question.)
>
> Class 3. Rejectable: Shucked oysters with a coliform MPN of 160,000 or more per 100 ml., and/or a Standard Plate Count of 1,000,000 or more per ml.

It was recommended at the Workshop that these criteria be applied by the shellfish control agencies in the receiving areas for an interim period. It was proposed that during this period studies be conducted to evaluate the applicability of the tentative standards. It was also

recommended at the 1956 Shellfish Sanitation Workshop that serious study be made of the validity of the coliform group and the Plate Count as indices of bacteriological condition of market oysters and furthermore that investigations should be initiated to determine the validity of other bacterial indices. E. coli was suggested as possibly the organism which should receive the most attention.

A short time later representatives of state, federal, and municipal control agencies met in Washington to outline a plan of study to evaluate these market criteria. The group included representation from Maryland, Virginia, New Jersey, New York State and New York City and the shellfish control and research sections of the Public Health Service. The plan of investigation formulated by that group involved the collection and examination of oysters from representative shucking and repacking establishments by the control agencies in the producing area and the simultaneous collection and examination of shucked oysters from these same sources by shellfish control agencies in the consuming area. Examinations were to be conducted for coliform MPN, Standard Plate Count, and E. coli MPN following procedures which were agreed to and outlined by the Shellfish Sanitation Laboratory. A copy of these procedures is incorporated in this report as Appendix I.

This study proceeded throughout almost two complete oyster harvesting seasons. The results obtained by all of the cooperating agencies were collected and summarized by the Public Health Service, Shellfish Sanitation Laboratory following procedures approved by Dr. E. K. Harris, Analytical Statistician, Sanitary Engineering Center. The results of this study were considered by the participating agencies at a meeting held in Washington, D. C. on June 26, 1958.

RESULTS

Conformity With Tentative Market Standards

The States of Maryland and Virginia examined more than 300 samples of oysters at various stages of harvesting, shucking, and repacking. Of these samples the data collected by the State of Virginia includes 95 sets of samples of shucked oysters as packed in Virginia shucking houses. The data collected by the State of Maryland includes 56 samples of shucked oysters as received from Virginia by Maryland repacking houses and 37 samples of these oysters after blending, washing, and repacking. New York City has presented data on the examination of 39 samples received from these repacking establishments, but in all New York City has submitted results of the examination of almost 300 samples of shucked oysters as received at the New York market, 196 of these having been received from Virginia and Maryland sources and 98 from New Jersey, Delaware and Connecticut sources. In addition the New York State Conservation Department has submitted the results of the examination of 27 samples from the two Maryland repackers under investigation. The Food and Drug Directorate of the Department of National Health and Welfare has submitted results on 66 samples of shucked oysters examined on receipt in the Canadian market.

All of the samples collected at the same stage in processing and marketing were submitted to a summarization procedure which utilizes log probability plots in accordance with the method first described by Velz(1) and later by Thomas(2). Preliminary frequency distributions of the data on a year round basis indicated a high degree of variation. Sorting the data by months revealed that in general higher values were obtained during the months of April, September, October and November than during the months of December, January, February and March. When the number of samples allowed subdivision into these two seasonal groups separate probability plots were prepared.

Coliform MPN's and Plate Counts of oysters at three probability percentiles are shown in Tables 1 and 2. Values obtained at the two selected seasons are shown as well as for all of the data collected throughout the entire harvesting season. These two tables are presented primarily to show the bacterial changes taking place in the various stages of processing and shipment. It can be noted that in all instances a definite seasonal variation was found. Both the coliform MPN and the Plate Counts are significantly higher during the warmer periods of the oyster harvesting season than during the colder months. It can also be noted that significant changes take place not only during handling but also on shipment. The rises during shipment are most likely a reflection of deficiencies in refrigeration practices.

To illustrate the extent of compliance with limits for coliform MPN and Plate Count suggested at the 1956 Shellfish Sanitation Workshop, the data contained in Tables 3 and 4 are presented. These show percent attainment at the various stages of processing and should be of assistance in estimating the reasonableness of the current tentative criteria of quality. MPN 230 was included primarily to illustrate the degree of conformity of the shell stock with the limit suggested in the

revision of Part I of the Public Health Service Manual. It has much less significance when applied to shucked oysters. Plate Count of 5,000 and 10,000 were included merely to show the degree of conformity with these arbitrarily selected limits to give some idea of the range of the Plate Count obtained at various stages.

The data indicates a high degree of compliance with the recommended market standards during the colder months. Eighty percent of the Maryland and Virginia oysters as received at the markets were within the acceptable class as judged by either coliform MPN or Plate Count, ninety-seven percent were either acceptable or acceptable on condition. During the warmer periods, however, compliance was much lower, particularly in the acceptable class. Only 56 percent of the Maryland and Virginia oysters were coliform MPN 16,000 or less.

The influence of temperature can also be seen by comparison of the data for Maryland and Virginia oysters with those from more northern sources. In both seasons the percentage compliance with coliform limits is much higher in the northern oysters. The effect of temperature can also be seen in the lower percentage compliance in northern oysters during the warmer period.

Relationships Between Coliforms and E. Coli

The bacteriological data presented at the Shellfish Sanitation Workshop suggested very strongly that the coliform group was not an entirely satisfactory index of quality of shucked oysters at the market level. The fact that the tolerances for coliform MPN's were relaxed in the tentative market standards is proof of this. In many of the presentations at the Workshop there appeared the suggestion that serious consideration should be given to a frank evaluation of the relative significance of the coliform group and E. coli as measures of sanitary condition of shucked oysters. The agencies cooperating in this study therefore decided to incorporate the determination of E. coli MPN in the program of evaluation of the tentative market standards.

The first stage in the investigation of the significance of E. coli was the preparation of a set of procedures for the enumeration of these organisms. There are no standard methods for this test either in Standard Methods for the Examination of Water, Sewage and Industrial Waste or in the Recommended Procedures for the Bacteriological Examination of Shellfish Waters and Shellfish. Lack of uniformity in procedures is probably one of the most important interferences with the acceptance of tests for E. coli as an index of sanitary quality. Variations from one laboratory to another hardly allow comparison of results obtained. The group therefore developed a procedure to be followed in this study.

The procedure is a liquid confirmatory method applied to all gas positive presumptive broth tubes using Perry and Hajna's(3) E.C. medium incubated at elevated temperatures. The detailed procedures followed in the study are described in Appendix I. Recognizing the lack of information concerning the reliability of the procedure, a plan of self evaluation was incorporated. This evaluation involves application of the completed-IMViC test to all gas positive E.C. tubes, and further, a completed-IMViC test on the LST presumptive tubes from which the E. C. tubes were transferred.

A summary of the results obtained are presented in Table 5. These are arranged somewhat in the same form as those for coliform MPN and Plate Count data and compare, on the same lot of samples, coliform MPN's with E. C. Medium gas positive and E. C. completed test MPN's at two probability percentiles. MPN's based on the presence or absence of gas in the E. C. tube are presented to show what might be expected if the partially confirmed test were applied.

Seasonal variations in both E. C. gas positive MPN and E. coli completed MPN were found, although not as great as with coliform MPN's. To some extent the higher MPN's may be the result of multiplication, but at least part of the seasonal difference may be attributable to increased activity of the oysters in warmer waters.

A comparison of the E. coli MPN's, both gas positive and completed test at the different stages shows little change on passage through the handling and shipping processes. The following table illustrates this.

E. COLI COMPLETED MPN'S AT 90 PERCENTILE

All Seasons

As Packed	As Received At Repackers	As Repacked	Market N.Y. City	Market N.Y. State	Market Canada
150	190	120	230	24	300

E. C. gas positive MPN's follow a similar pattern.

Table 6 is presented to show the frequency of occurrence, at the different stages in processing and shipment, of four arbitrarily selected MPN values. A characteristic of the E. coli results obtained is the high frequency of very low or indeterminate values. For example, 62 percent of the samples of Maryland and Virginia oysters sampled by New York City showed E. coli completed test MPN's of less than 18 and 75 percent showed MPN's 20 or less. Two-thirds of these samples showed E. C. gas positive MPN's of 20 or less. With the exception of New York State E. coli completed MPN's, the results obtained by other agencies were quite similar.

The data in Table 6 also shows that between 88 and 96 percent of all samples of shucked oysters at the market were E. coli completed test MPN 230 or less and between 84 and 96 percent of the same samples were E. C. gas positive MPN 230 or less.

Relationships Between E. C. Gas Positive and E. Coli Completed Test

The E. C. completed test, because it involves classification by IMVIC is extremely demanding of time and materials. Minimum time for obtaining a completed test result is nine days after the initial inoculation in the presumptive broth. If the partially confirmed test E. C. test results could be applied with a reasonable degree of reliability, most of the time results would be obtained in three days, and occasionally four to five days. The E. C. liquid confirmatory procedure is no more involved than the liquid confirmatory test for coliform organisms.

A comparison of the E. C. gas positive and E. C. completed test MPN's shown in Table 6 shows that the disagreement between these two methods is largely in the lower MPN ranges. To what extent this is a true relationship and not experimental error, both in the test and in the method of enumeration is difficult to determine without a complete statistical analysis and probably basic experimentation. But the data shows a gradually decreasing difference between E. C. gas positive and E. C. completed test on approach to MPN 230. At that MPN level, the difference in percent occurrence of E. C. gas positive and E. coli completed test in market oysters listed in Table 6 is 3, 4, 0, and 3, in the order of their appearance in Table 6.

To demonstrate the variations obtained in E. C. gas positive MPN's at the lower levels of E. coli completed test MPN's, the following table is presented.

E. C. GAS POSITIVE MPN'S OBTAINED AT E. COLI COMPLETED TEST MPN <18 & 20

E.C.Gas Positive	Number of Samples						All Agencies	
MPN	Va.	Md.	N. Y. State	N.Y. City	Canada	Number	Actual%	Accumulated%
<18	32	37	13	60	13	155	66.5	66.5
20	11	11	5	20	3	50	21.5	88.0
45	3	2	4	5	1	15	6.4	94.4
78			1	5		6	2.5	96.9
130			1	4		5	2.1	99.0
230					1	1	0.4	99.4
330					1	1	0.4	99.8
2,400				1		1	0.4	100
Tot.Samp.	46	50	24	95	19	234		

Again, the reality of these figures can be revealed only by statistical analysis to differentiate between real differences and inherent experimental error. But the data as presented shows that at the E. coli completed MPN level <18 to 20 almost 95 percent of the samples were in agreement if a latitude of two tubes planted were considered and that 99 percent of the samples showed gas positive MPN's 130 or less when the completed test MPN was <18 or 20.

Falsely Positive E. C. Partially Confirmed Tests

The E. coli procedure followed in the cooperative study included a plan for self-evaluation. Falsely positive E. C. partially confirmed tests (gas production in E. C. medium) were revealed by the failure to recover E. coli in the completed-IMViC test performed on the gas positive E. C. tubes.

From the data available to him at the time, Dr. Harris has computed the "apparent percent falsely positive" E. C. partially confirmed tests. Values of 27, 39, and 50 percent were obtained on data submitted by three of the participating agencies. For comparison, he also computed values of 33 percent, 34 percent and 8 percent for data collected on the Gulf Coast.

These variations in percentage false positives are not unique to this study. Investigations at the Sanitary Engineering Center(4) on cultures of coliforms received from 14 geographical areas have shown a definite variation, associated with geographical area, in the ability of non-E. coli types to produce gas in E. C. medium at 45.0°C. Similar behavior was found on the use of Boric Acid Lactose Broth.(5)

By far the most frequent IMViC coliform type responsible for the falsely positive tests in the cooperative study was Aerogenes type 1 (IMViC type --++). Next in order of occurrence, but not necessarily in order of ability to produce gas in E. C. medium was E. coli type 2 (IMViC type -+--). Stuart et al, (6) in investigations of the Eijkman reaction of coliforms isolated from various sources found that these were the only coliform types other than E. coli type 1 showing ability to produce gas in Eijkman broth in a water bath at 45.5°C. Stuart found that about 2 percent of 680 cultures IMViC type --++ produced gas and about 85 percent of 134 IMViC type -+-- cultures produced gas at 45.5°C. In the work at the Sanitary Engineering Center 10.6 percent of the IMViC type --++ and 21.8 percent of the IMViC type -+-- produced gas in E. C. medium at 45°C.

The specificity and constancy of the test is also greatly influenced by temperature of incubation. All investigators of the test have established very close tolerance for temperature of incubation, usually ± 0.2°C. The procedures for this study had detailed stipulations for temperature and condition of incubation. Reduction in incubation temperature of as little as 0.5°C. has been found by participants in this project to have caused significant differences in the numbers of false positive tubes.

DISCUSSION

In a cooperative study undertaken by nine shellfish control agencies samples of oysters have been examined for coliform MPN, E. C. gas positive MPN, E. coli completed test MPN and Standard Plate Count, in an attempt to evaluate (1) the applicability of the tentative market standards for shucked oysters adopted at the 1956 Shellfish Sanitation Workshop; (2) the relative significance of the coliform group and E. coli as measures of sanitary condition of market shucked oysters. The project included the examination of samples of shell and shucked oysters at all stages of handling and shipment from the shell stock before shucking to the final product as received in the consuming areas. The project continued throughout two complete shellfish harvesting seasons.

The results obtained during the course of this cooperative study were discussed at a meeting held on June 26, 1958 and attended by representation from all of the participating agencies with the exception of the Food and Drug Directorate of Canada. It was the recommendation of that group, after a review of a detailed summary of the results obtained, that a report on the study be prepared for presentation at the 1958 Shellfish Sanitation Workshop.

The results obtained indicate a high degree of compliance with the coliform MPN stipulations of the tentative market standards in all sections of the study area. A definite seasonal and geographical difference was observed however. Comparison of the results obtained on shucked oysters received from sources in Maryland and south, showed considerably higher coliform MPN's than those received from the more northern areas. This would suggest that the present coliform MPN limits are difficult to apply since the significance of these high values in the north are much greater than in the more southern areas.

Compliance with the Plate Count limits was equally as good in oysters obtained from all areas. There was an indication, however, that the Plate Count of less than one million as the upper limit for the "Acceptable on Condition" class is too liberal. The data suggested that a limit of less than 500,000 would enclose a more reasonable number of samples. MPN's obtained by both E. C. gas positive test and the E. coli completed test appear to show considerable less variation in oysters throughout the region under study. As an index of pollution or fecal contamination, therefore, the use of this type of test would appear to be more suitable once the reliability of the procedure and its inherent experimental errors are determined.

The E. C. results, both gas positive MPN and completed test MPN, are characterized by a high percentage of low or indeterminate values. This together with the fact that the greatest difference between MPN's obtained by these two tests on the same samples were found to be at low MPN values, would suggest considerable latitude in the limits set for assessment of quality. Sample to sample comparisons of MPN's obtained by these two methods show that there is little significant difference within the first three 1cc portions planted.

Preliminary estimates have been obtained of the reliability of the E. C. partially confirmed test as an index of the presence or absence of E. coli. "Apparent False Positive Percentages" computed for the data submitted by individual laboratories shows a considerable variation from one laboratory to another. The significance of these differences in error of the test is reduced to some degree by the fact that the majority of these differences occur at low MPN values.

After consideration of the summaries of the results obtained the group which met in Washington on June 26 arrived at the following conclusions and recommendations:

1. It was concluded by the group that bacteriological criteria of sanitary quality as applied to shucked oysters in the market serves a useful purpose.

2. The Standard Plate Count serves as a useful index of unsatisfactory handling during processing and/or improper refrigeration or mishandling while the product is in transit.

3. The coliform MPN is not much more meaningful as a criterion of quality than the Standard Plate Count.

4. Serious consideration should be given to the substitution of E. coli for coliforms as a bacterial index of market quality of shucked oysters.

REFERENCES

1. Velz, C. J. 1951: Graphical Approach to Statistics - Evaluation of Bacterial Densities. Water and Sewage Works 98, 66-73

2. Thomas, Harold A. 1955: Statistical Analysis of Coliform Data. Sewage and Industrial Wastes. 27, 212-222

3. Hajna, A. A., and Perry, C. A. 1943: Comparative Study of Presumptive and Confirmatory Media for Bacteria of the Coliform Group and for Fecal Streptococci. Am. J. Pub. Health, 33, 550-556.

4. Geldreich, E. E., Clark, H. F., Kabler, P. W., Bordner, R. H., and Huff, C. B.: The Coliform Group: II. Reactions of Coliform IMViC Types in a Modified EC Test at 45.0°C. Incubation. Presented at the 85th Annual Meeting of the American Public Health Association November 1957. To be published in Applied Microbiology.

5. Clark, H. F., Geldreich, E. E., Kabler, P. W., Bordner, R. H., and Huff, C. B. 1957: The Coliform Group: I. The Boric Acid Lactose Broth Reaction of Coliform IMViC types. Applied Microbiology, 5, 396-399.

6. Stuart, C. A., Zimmerman, Alice, Baker, Muriel, and Rustigian, Robert 1942: Eijkman Relationships of the Coliform and Related Bacteria. Jour. Bact. 43, 557-572.

TABLE I

COLIFORM MPN AT 10, 50, AND 90 PROBABILITY PERCENTILES

SHELL AND SHUCKED OYSTERS

Percentile	COLIFORM MPN								
	Shucking		Maryland Repacking		New Jersey Repacking		Market		
	Shell Stock	As Packed	As Rec'd.	As Repacked	As Rec'd.	As Repacked	Md. Repacker	All Md. & Va. Packers	Packers N. of Md.
				Dec.-Jan.-Feb.-March					
10	18	150	940	480	320	40	1,100	160	<18
50	240	1,400	7,200	5,000	6,600	520	9,200	2,800	130
90	3,800	14,000	54,000	52,000	13,000	8,000	75,000	50,000	22,000
No. of Samples	39	50	38	25	25	23	31	139	93
				Apr.-Sept.-Oct.-Nov.					
10	100	920	2,400	1,500	---	---	---	2,000	80
50	2,000	11,000	19,000	6,400	---	---	---	13,000	3,500
90	35,000	110,000	140,000	26,000	---	---	---	80,000	140,000
No. of Samples	21	45	18	12	---	---	8	66	26
				All Seasons					
10	16	270	1,100	800	---	---	1,700	230	<18
50	490	3,500	10,000	5,300	---	---	11,000	4,100	390
90	15,000	44,400	90,000	35,000	---	---	76,000	74,000	39,000
No. of Samples	60	95	56	37	---	---	39	205	119

TABLE 2

STANDARD PLATE COUNTS AT 10, 50, AND 90 PROBABILITY PERCENTILES

SHELL AND SHUCKED OYSTERS

Percentile	STANDARD PLATE COUNT						
	Shucking		Maryland Repacking			Market	
	Shell Stock	As Packed	As Received	As Repacked	Maryland Repackers	All Md.&Va. Packers	Packers N. of Md.
			Dec.-Jan.-Feb.-March				
10	100	960	2,600	<3,000	1,900	1,400	800
50	800	5,500	14,000	4,900	27,000	15,000	7,000
90	6,700	31,000	76,000	100,000	260,000	160,000	150,000
No. of Samples	34	50	38	25	30	99	99
			Apr.-Sept.-Oct.-Nov.				
10	140	1,800	8,600	---	---	2,600	1,000
50	2,500	17,000	36,000	---	---	22,000	7,000
90	45,000	150,000	140,000	---	---	190,000	170,000
No. of Samples	20	45	18	12	8	98	27
			All Seasons				
10	120	1,100	3,200	1,100	4,000	1,500	900
50	1,200	7,600	19,000	8,600	30,000	16,000	7,000
90	12,000	70,000	110,000	70,000	220,000	170,000	160,000
No. of Samples	54	95	56	37	38	197	126

TABLE 3

PERCENT ATTAINMENT OF MPN LIMITS

SHELL AND SHUCKED OYSTERS

	PERCENT ATTAINMENT								
	Shucking		Maryland Repacking		New Jersey Repacking			Market	
MPN	Shell Stock	As Packed	As Rec'd.	As Repacked	As Rec'd.	As Repacked	Md. Repacker	All Md.&Va. Packers	Packers N. of Md.
				Dec.-Jan.-Feb.-March					
230	48	15	---	---	---	---	---	---	---
2,400	86	62	24	34	72	72	20	48	75
16,000	97	91	69	74	92	95	63	80	90
<160,000	99.9	99.7	97.5	96	99	99.9	96	97	97
No. of Samples	39	50	38	25	25	23	31	139	93
				Apr.-Sept.-Oct.-Nov.					
230	18	2	---	---	---	---	---	---	---
2,400	54	21	10	20	---	---	11	12	44
16,000	82	59	47	80	---	---	47	56	80
<160,000	97.5	92	91	99.9	---	---	91	96	91
No. of Samples	21	45	18	12	---	---	8	66	26
				All Seasons					
230	39	8	---	---	---	---	---	---	---
2,400	72	42	20	29	---	---	15	40	70
16,000	91	78	61	77	---	---	60	72	85
<160,000	98.5	97	95	93	---	---	96	95	96
No. of Samples	60	95	56	37	---	---	39	205	119

TABLE 4

PERCENT ATTAINMENT OF PLATE COUNT LIMITS

SHELL AND SHUCKED OYSTERS

Standard Plate Count	PERCENT ATTAINMENT						
	Shucking		Maryland Repacking			Market	
	Shell Stock	As Packed	As Received	As Repacked	Maryland Repackers	All Md.&Va. Packers	Packers N. of Md.
			Dec.-Jan.-Feb.-March				
5,000	87	46	---	---	---	---	---
10,000	94	68	40	---	28	40	56
50,000	99.4	95	83	---	64	74	77
500,000	---	99.9	---	---	96	97	96
<1,000,000	---	---	99.9	---	98	99	98
No. of Samples	34	50	38	---	30	139	99
			Apr.-Sept.-Oct.-Nov.				
5,000	62	24	---	---	---	---	---
10,000	73	38	12	---	---	32	57
50,000	91	74	62	---	---	70	78
500,000	99.1	98	---	---	---	97	96
<1,000,000	---	99.9	99.9	---	---	99	98
No. of Samples	20	45	18	---	8	139	27
			All Seasons				
5,000	78	37	---	---	---	---	---
10,000	88	54	32	54	25	40	56
50,000	98	86	77	86	63	72	77
500,000	---	---	99.2	99.5	96	97	96
<1,000,000	---	---	99.8	99.8	98.6	99	98
No. of Samples	54	90	56	37	38	205	126

TABLE 5

COLIFORM E. C. GAS POSITIVE AND E. C. COMPLETED TEST MPN'S

Shucked Oysters At Various Stages of Processing

Stage in Process	MPN					
	Coliform		E.C. Gas Positive		E.C. Completed	
	50%	90%	50%	90%	50%	90%
As Packed						
All Year	700	19,000	<18	170	<18	150
As Received						
Summer	20,000	120,000	63	1,000	27	360
Winter	5,500	66,000	<18	130	<18	88
All Year	7,200	66,000	<18	300	<18	190
As Repacked						
All Year	4,600	27,000	<18	320	<18	120
Market						
New York City						
Md. and Va.						
Summer	16,000	160,000	30	1,100	<18	660
Winter	6,800	90,000	<18	100	<18	50
All Year	11,000	120,000	<18	330	<18	230
New York State						
2 Md. Packers	2,600	21,000	<18	90	<18	24
All Sources	1,400	18,000	<18	240	<18	94
Canada						
All Samples						
North & South	3,500	400,000	<18	360	<18	300
New York City						
North of Md.						
All Year	2,800	68,000	<18	490	<18	350

TABLE 6

PERCENT ATTAINMENT OF E. C. GAS POSITIVE AND E. COLI COMPLETED TEST MPN'S

	Percent Attainment						
	Repacker			Market			
MPN	As Packed	As Received	As Repacked	New York City Md.&Va.	New York City N. of Md.	N.Y. State	Canada
			E. C. Gas Positive				
<18	59	54	47	50	49	46	43
20	78	60	53	66	62	68	57
230	94	88	87	87	84	96	86
1,600	96	97	97	95	94	99+	97
			E. Coli Completed				
<18	63	59	63	62	65	86	50
20	84	69	71	75	69	90	63
230	94	92	94	90	88	96	89
1,600	96	98	98	96	96	99+	97

APPENDIX I

COLLABORATIVE SURVEY OF BACTERIOLOGICAL CONDITION OF MARKET OYSTERS

PROCEDURES FOR THE BACTERIOLOGICAL EXAMINATION OF OYSTERS

1. Collection and Transportation of Samples

Samples of oysters should be examined within six hours after collection and in no case shall they be examined if they have been held more than 24 hours. The report of examination shall include a record of the time elapsed between collection and examination.

Individual containers of shellfish samples shall be marked for identification and the same mark inserted in its proper place on the descriptive form which accompanies the sample.

A. Shell Stock

In general, twelve or more oysters, judged to be representative of the lot to be sampled shall be selected. The oysters selected shall have unbroken lips and the shells tightly closed. The number of oysters collected shall be sufficient to produce not less than 200 grams of meats and shell liquor.

Samples of oysters shall be collected in sterilized containers. Suitable containers for the purpose are a can with lid or a waterproof bag. The samples shall be kept in dry storage at a temperature between 0°C. and 10°C. until examined.

B. Shucked Oysters

Samples of shucked oysters may be taken in the original container, if of suitable size; or the oysters may be transferred to a sterile wide mouth jar, using sterile forceps or spoon. Not

less than 200 grams (approximately $\frac{1}{2}$ pint) shall be taken as a sample. The sample shall be refrigerated immediately after collection by packing in crushed ice and kept at the temperature of melting ice until examined.

C. Frozen Shucked Oysters

If the package contains 16 ounces or less, one or two packages may be taken as a sample. Samples from larger blocks may be taken by coring with a suitable instrument or by quartering, using sterile technic. The sample shall be 200 grams or more. Cores or quartered samples shall be transferred to sterile wide mouth jars for transportation to the laboratory.

II. Preparation of Sample for Examination

A. Shell Oysters

1. Cleaning the Shells

Before starting to clean the shells, the hands of the examiner shall be thoroughly scrubbed with soap and water.

Scrape off all growth and loose material from the shell and scrub the oysters with a sterile stiff brush under running water paying particular attention to the crevices at the junctions of the shells. The water shall be from a supply approved by the appropriate state agency as a drinking water supply. Place the cleaned oysters in clean containers or on clean towels and allow to drain in the air.

2. Removal of Shell Contents

Before starting the removal of shell contents, the hands of the examiner shall be thoroughly scrubbed with soap and water and rinsed with 70 percent alcohol.

Hold the oyster in the left hand, with the deep shell on the bottom and the hinge pointed away from the examiner. Using a sterile oyster knife, insert the point between the shells on the ventral side (at the right of the examiner) about one-fourth the distance from the hinge to the bill. Entry may also be made at the bill after making a small opening with an instrument similar to bone cutting forceps, suitably sterilized.

Cut the adductor muscle from the upper flat shell and pry the shell wide enough to drain the shell liquor into a sterile tared beaker or wide mouth jar. The upper shell may then be pried loose at the hinge, discarded, and the meats then transferred to the beaker or jar after severing the muscle attachment to the lower shell.

Open a sufficient number of oysters to obtain approximately 200 grams of meats and shell liquor, except that not less than six oysters shall constitute a sample.

B. Shucked Oysters

Transfer approximately 200 grams of oysters from the sample jar to a sterile tared beaker or jar, using sterile forceps of spoon.

C. Dilution and Grinding

Weigh the sample to the nearest gram.

Transfer the weighed sample to a sterile grinder and add an equal amount by weight of sterile phosphate buffered dilution water.

Grind for 90 seconds in a blendor similar to a Waring Blendor operating at 35,000 RPM.

III. Examination for Members of the Coliform Group

A. Inoculation

The ground sample should be inoculated immediately. If there is any delay, it shall be thoroughly mixed immediately before inoculation.

Lauryl tryptose broth, single strength in fermentation tubes, shall be used as the presumptive test medium. Dilutions shall be made in sterile phosphate buffered dilution water.

Inoculate:

1. Each of 5 tubes with 2 ml. of prepared sample. (Equal to 1 gram of oysters.)
2. Each of 5 tubes with one ml. of a 2 to 8 dilution of prepared sample. (Equal to 0.1 gram of oysters.)
3. Each of 5 tubes with one ml. of a 1 to 9 dilution of the dilution prepared in (2) above. (Equal to 0.01 gram of oysters.)
4. Each of 5 tubes with one ml. of a 1 to 9 dilution of the dilution prepared in (3) above or one-tenth ml. of the dilution prepared in (3). (Equal to 0.001 gram of oysters.)

In order to avoid indeterminate results, extension of the dilutions suggested above may be necessary. The amounts of sample selected for inoculation should be such that the largest portions will give positive results in all or the majority of tubes and the smallest portions will give negative results in all or the majority of tubes. To attain this with any degree of assurance with samples of doubtful quality it will be necessary to inoculate four decimal dilutions.

B. Presumptive Test

Incubate the fermentation tubes at 35°C. ± 0.5°C. Examine each tube at the end of 24 ± 2 hours and if no gas has formed, again at the end of 48 ± 3 hours. Formation of gas may be recognized by the presence of visible gas in the upper end of the inverted fermentation tube or the presence of gas bubbles throughout the medium outside the fermentation tube when gently shaken.

Formation of gas within the stipulated incubation periods constitutes a Positive Presumptive Test which must be confirmed.

The absence of gas formation after 48 hours incubation constitutes a Negative Test for coliform group organisms.

C. Confirmed Test for Coliform Group Organisms

All presumptive test positive fermentation tubes at the end of 24 hours or 48 hours incubation shall be subjected to the confirmed test. If active fermentation appears in the primary fermentation tube before the expiration of the 24 hour period it is preferable to transfer these cultures without waiting for the completion of the full period.

In routine examinations, where the proportion of false positive presumptive tubes is known to be low, confirming all tubes may not be necessary. Sufficiently accurate results may be obtained if confirmation is conducted on the tubes of only the two highest dilutions showing gas when all five tubes of the lower dilution are gas positive. However, in the event of "skips", confirmation should be conducted on all tubes in the dilutions showing less than five tubes positive. (A detailed discussion on this subject will be found in "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes", Tenth Edition, p. 376.)

1. Transfer a loopful (loop not less than 3mm diameter) of culture from the presumptive tube to a fermentation tube containing brilliant green lactose bile broth.
2. Incubate the inoculated tubes for 48 ± 3 hours at 35° ± 0.5°C.
3. Formation and presence of gas in any amount in the inverted vial of the confirmatory tube within 48 hours constitutes a Confirmed Test for coliform group organisms.

D. Expression of Results

The coliform group density shall be expressed as "Most Probable Number" (MPN) per 100 grams of sample. MPN tables and a discussion of the method of computing the MPN will be found in "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes", Tenth Edition pp. 382-387.

IV. Standard Plate Count

A. Plating

In preparing the plates, such amounts of sample should be planted as will give from 30 to 300 colonies per plate and the aim should be to have at least two plates giving colonies between these limits.

1. Add the appropriate amount of sample, using the dilutions prepared for the inoculation of the presumptive tubes, to the petri dish. Usually 0.1 and 0.01 gram amounts of shell and shucked oyster samples taken in shucking plants and 0.01 and 0.001 gram amounts of shucked oysters sampled in the market will produce plates of the desired density.

2. Add not less than 10 ml. of liquified tryptone glucose extract agar at a temperature of 43° to 45°C. to the inoculated dish. Not more than 20 minutes should elapse between plating and pouring. Mix the medium and sample by tilting and rotating the dish.

3. Incubate the solidified plates at 35° ± 0.5°C. for 48 hours.

B. Counting

Counting shall be done with an approved counting aid, such as the Quebec Colony Counter.

C. Expression of Results

The count shall be expressed as Standard Plate Count per gram of sample. The procedure for computing the Standard Plate Count as well as complete details concerning the entire Standard Plate Count procedure will be found in "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes", Tenth Edition, pp. 373-374, and in "Standard Methods for the Examination of Dairy Products", Tenth Edition, pp. 90-113.

V. Estimation of the Density of E. Coli in Oysters

A. Procedure

The relative density of *E. coli* in both plant and market samples being unknown but most likely much lower than that of the coliform group will require submitting all gas positive presumptive tubes to the liquid confirmatory procedure for *E. coli*. Therefore, until the procedure has been evaluated and the coli-coliform relationships have been determined, the scheme of eliminating certain of the gas positive tubes in the lower dilutions as outlined in the confirmed test for coliform organisms (III-C of the Recommended Procedures) shall not be followed in this test for *E. coli*.

In view of the many divergent opinions concerning the significance of gas production in the confirmatory media as an indication of the presence or absence of *E. coli*, confirmatory procedures are recommended until experience has demonstrated the reliability of the procedure. All gas positive liquid confirmatory tubes shall be submitted to a completed-IMViC procedure as shown in the attached outline. To determine the number of false negative reactions in the liquid confirmatory broth the procedure recommends parallel completed-IMViC tests at 35°C. conducted directly from the gas positive presumptive broth tubes.

The procedure recognizes two methods of incubation; water bath at 44.5°C. ± 0.2°C. and air incubation at 45.5°C. ± 0.2°C. Tests have demonstrated that water bath and air bath incubation produce different patterns of temperature attainment in the culture medium. The least lag in attainment of temperature was accomplished in a water bath equipped with a stirring device.

In view of the evidence that air incubation at 45.5°C. in a well designed and well operated air incubator will produce acceptable results, this method is recognized as an alternate provided that a uniform lag period and temperature control is possible throughout the incubator under all conditions of loading the incubator. With some incubators this might require forced draft circulation.

The participating laboratories shall periodically check and record the temperature attainment in the culture medium and the uniformity of attainment throughout the incubator. The

thermometers used for this purpose shall be checked for accuracy at the incubation temperature against a thermometer standardized by the Bureau of Standards. The thermometer scale should be subdivided to at least 0.2°C. preferably 0.1°C.

1. Transfer a loopful (loop not less than 3mm diameter) of culture from the gas positive fermentation tube (lauryl tryptose broth) to a fermentation tube of the liquid confirmatory medium (E.C. medium).

Incubate the inoculated liquid confirmatory tubes in a water bath at 44.5°C. ± 0.2°C. or in an air incubator at 45.5°C. ± 0.2°C. The temperature stipulated shall be that of the culture medium.

2. Streak one or more plates of EMB agar with culture from the same gas positive presumptive tubes. Incubate the EMB plates at 35°C. ± 0.5°C. for 24 ± 2 hours.

3. From each of the EMB plates streaked from the presumptive tubes fish two typical coliform colonies to lactose broth fermentation tubes. If no typical coliform colonies are present, fish two or more colonies considered most likely to consist of organisms of the coliform group. Incubate the lactose broth tubes at 35°C. until gas formation is noted, the incubation not to exceed 48 hours.

Purify the cultures by successive transfer to lactose broth and to EMB agar if necessary, and, when the purity of the culture has been established, continue with the differentiation by the IMViC procedure as outlined in "Standard Methods for the Examination of Water, Sewage and Industrial Waste", Tenth Edition, pp. 390-393.

4. Examine the liquid confirmatory tubes for gas production at the end of 24 ± 2 hours and, if no gas has formed, again at the end of 48 ± 3 hours.

5. Streak one or more plates of EMB agar with cultures from each of the gas positive liquid confirmatory tubes.

Incubate the EMB plates at 35°C. ± 0.5°C. for 24 ± 2 hours.

From each of the EMB plates streaked from the liquid confirmatory tubes fish two typical coliform colonies to lactose broth fermentation tubes. If no typical coliform colonies are present, fish two or more colonies considered most likely to consist of organisms of the coliform group. Incubate the lactose broth tubes at 35°C. until gas formation is noted, the incubation not to exceed 48 hours.

Continue with the differentiation by IMViC procedure as outlined in item 3 above.

B. Expression of Results

Production of gas in the liquid confirmatory tube constitutes a Positive Partially Confirmed Test for E. coli. Demonstration of the presence of E. coli types 1 or 2 by the IMViC differentiation constitutes a Positive Completed Test.

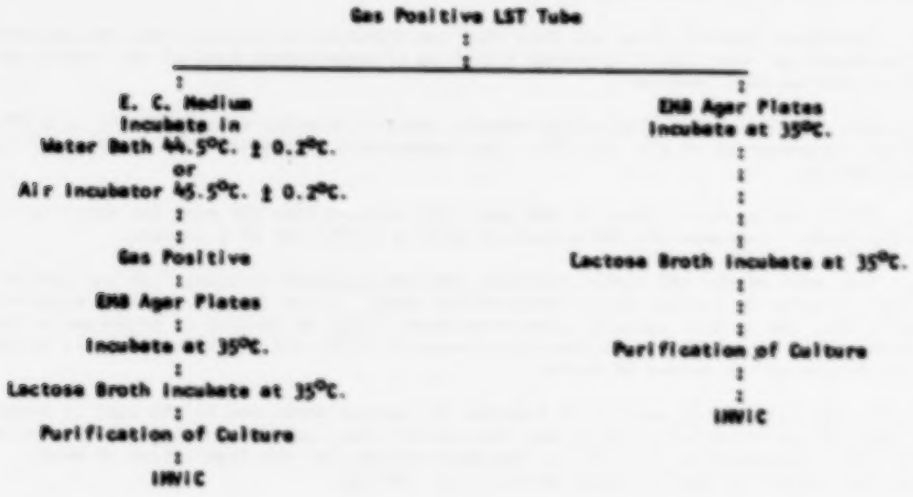
MPN's shall be computed from the positive and negative tube pattern of the completed test results. The report of the results should include a statement of the type and temperature of incubation.

VI. Media and Glassware - Composition and Preparation

Procedures for the preparation of culture media and dilution water, and sterilization of glassware shall be in conformity with the methods and formulae described in "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes", Tenth Edition pp. 362-373.

For the purpose of obtaining greater uniformity in results, however, the participants in the co-operative survey shall use dehydrated culture media exclusively.

LIQUID CONFIRMATORY PROCEDURE FOR THE ENUMERATION OF E. COLI



VII. Participating Agencies

The following agencies participated in the study: Department of National Health and Welfare of Canada, Food and Drug Directorate; Maryland State Department of Health, Bureaus of Environmental Hygiene and laboratories; New Jersey State Department of Health, Bureaus of Food and Drugs and Laboratories; New York City Department of Health, Bureaus of Food and Drugs and Laboratories; New York State Conservation Department, Bureau of Marine Fisheries (Shellfish Management Unit); U. S. Public Health Service, Division of Sanitary Engineering Services, Milk and Food Program and Sanitary Engineering Center (Shellfish Sanitation Laboratory, Milk and Food Research and Analytical Statistics Unit), and Regions II and III; and Virginia State Department of Health, Division of Engineering.

These procedures for the bacteriological examination of oysters were prepared by the Shellfish Sanitation Laboratory, U. S. Public Health Service, in collaboration with state agencies participating in the cooperative study.

APPENDIX B

RESULTS OF BACTERIOLOGICAL ANALYSIS OF IMPORTED U.S. SHUCKED OYSTERS, 1957-1958

By

Dr. F. S. Thatcher
Food and Drug Directorate
Department of National Health and Welfare
Ottawa, Canada

All samples analysed were collected in Montreal, packed in crushed ice and shipped to Ottawa for analyses. The bacteriological procedures used were those given the "Collaborative Survey of Bacteriological Condition of Market Oysters, Procedures for the Bacteriological Examination of Oysters," as prepared by the Shellfish Sanitation Laboratory, U.S. Public Health Service on September 10, 1957. All Eijkman confirmations were done in a water-bath incubator at 44.6°C. (culture temperature).

Results obtained from the analyses appear in the following tables. The last column of table II indicates which of the 3 provisional market grades (Class I - Acceptable; Class II - Acceptable on Condition; Class III - Rejectable) each of the samples falls into, together with the reason for placing it there. Of the 29 samples examined 15 were classed acceptable, 7 were acceptable on condition and 7 were rejectable. In these latter 2 classes 3 samples in each class were placed there on the basis of plate count alone. With them coliform content alone would have shifted their classification upwards by 1 class. The other 8 of the 14 Class II and Class III samples would have been graded identically either by standard plate counts or coliform MPN's, and indicate that present class limits for coliform content and plate count are more realistic and practical than the original limits set for these grades.

The Virginia pack oysters which in previous years caused considerable concern in that they included a high percentage of Class II and Class III shipments have shown improvement which is not explained only by the re-defining of class limits. There has been an obvious improvement in the bacteriological quality of these packs, as seen by the fact that 5 out of 6 of these packs had coliform MPN's well under the old class I limit of 2400.

We now see the same situation that used to occur with the Virginia packs repeating itself with a more northern packer (Robbins Bros., N.J.6). The first 5 shipments examined from this packer were Class III and of a total of 12 shipments examined 6 were Class III, 2 were Class II and 4 were Class I. The improvement occurred at the end of the season although even in the last 4 shipments examined 1 Class III and 1 Class II sample was found.

No definite pH change was noted in any of the samples until the plate count was approaching 10 million.

E. coli determinations: As suggested in the outline of procedure the presence of *E. coli* was checked for in all positive presumptive tubes both by direct plating on E.M.B. agar for isolation and by subculturing in Eijkman broth at elevated temperature and plating for isolation all positive Eijkman tubes. In the analysis of the first 25 samples, a total of 548 positive presumptive tubes were obtained. By direct plating procedures 27 tubes were found to contain *E. coli*. However subculture to Eijkman 44.6°C. yielded 40 positive tubes of which 37 were confirmed as containing *E. coli* following plating and identification via "imvic" reaction. Results for the last 4 samples are included in the table but their significance is questionable due to temperature variations in the incubator at this time. Cause of this temperature variation is not known but since daytime operation was satisfactory it is likely that power interruptions occurred during the night.

While Eijkman incubation temperatures remained constant, 92.5% of positive Eijkman tubes contained *E. coli*. Exceptions were; 1 of 3 Eijkman positive tubes in sample #3 contained *A. aerogenes* I and both Eijkman positives in sample #9 appeared to contain only NR + VP + organisms which produced very small amounts of gas in Eijkman broth. This last sample was also the only instance where *E. coli* was isolated from 1 positive presumptive tube and was not isolated from the 2 weakly positive Eijkman confirmatory broth tubes.

Including the 4 samples done when temperature variation was noted dropped the percentage of Eijkman positives containing *E. coli* from 92.5 to 75.8% and was due to the increased isolation of *A. aerogenes* I and intermediate I strains from these tubes. Provided that no temperature fluctuation difficulties are encountered the results indicate that the preferred method for *E. coli* determination is the Eijkman confirmation procedure. Not only is recovery higher than by direct isolation from presumptive tubes, but a considerable saving of time and materials is realized.

TABLE I

Results of Bacteriological Analysis of Shucked Imported U.S. Oysters, December 1957 to April 1958.

Sample Identification	Plate Count per gm.	Coliform MPN per 100 gms.	*E. coli* Determined Via: Presumptive Tubes — Positive Tubes	Presumptive Tubes — Confirmed *E. coli* Tubes	Presumptive Tubes — *E. coli* MPN per 100 gms.	Positive Eijkman Tubes — Positive Tubes	Positive Eijkman Tubes — Eijkman MPN per 100 gms.	Positive Eijkman Tubes — Tubes with Confirmed *E. coli*	Positive Eijkman Tubes — *E. coli* MPN per 100 gms.	pH
A-1	680	170	6	0	<18	0	18	-	-	6.25
B-1	2,500,000	92,000	19	8	330	9	1,100	9	1,100	6.20
C-1	760	1,100	10	0	<18	3	68	2	45	6.05
D-1	5,300	170	6	0	<18	0	<18	-	-	6.05
E-1	8,500,000	160,000+	20	2	45	2	45	2	45	5.7
B-2	8,000,000	160,000+	20	1	<18	2	37	2	37	5.7
D-2*	34,000	140	8	0	<18	0	<18	-	-	6.1
A-2	750	220	11	0	<18	0	<18	-	-	6.1
E-2	240,000	12,000	21	1	20	2	36	0	<18	6.2
F-1*	330,000	92,000	18	0	<18	0	<18	-	-	6.25
A-3	920	1,300	10	0	<18	0	<18	-	-	6.3
B-3*	96,000	92,000	18	3	68	5	140	5	140	6.3
C-1	17,500	790	11	0	<18	0	<18	-	-	6.4
E-3	79,000	61	3	0	<18	0	<18	-	-	6.3
B-3	1,100,000	170,000	20	6	220	7	490	7	490	6.2
A-4	710	83	4	0	<18	0	<18	-	-	6.25
B-4	1,700,000	48,000	22	1	20	1	20	1	20	6.1
E-4	4,300	11,000	14	1	20	1	20	1	20	6.2
D-4*	1,700	170	7	0	<18	0	<18	-	-	6.25
B-5*	3,350,000	180,000	22	1	20	4	110	4	110	6.25
A-5*	119,000	3,500	20	0	<18	0	<18	-	-	6.2
B-6	415,000	16,000	20	0	<18	0	<18	-	-	6.25
D-5	8,800	410	12	0	<18	0	<18	-	-	6.25
B-7	9,000	790	12	2	40	2	40	2	40	6.2
B-8	41,000	5,400	14	1	20	2	45	2	45	6.05
	Sub-Total		348	27		60		37		
B-9	10,000,000	35,000	21	0	<18	5	230	0	<18	5.7
B-10	3,100	1,300	12	2	45	4	130	3	78	6.2
B-11	390,000	92,000	19	4	92	7	260	7	260	6.1
B-12	11,000	1,400	19	0	<18	6	330	0	<18	6.3
*Select Oysters	Total		419	33		62		47		

TABLE II

Results of Bacteriological Analysis of Shucked Imported U.S. Oysters, December 1957 to April 1958.

Sample Identification Code	Packer		Date Fished	Date Analysed	Class and Reason
	Name	No.			
A-1	Ballard	Va 207	5/12/57	11/12/57	I
B-1	Robbins Bros.	NJ 6	3/12/57	11/12/57	III SPC
C-1	Watson	Va 283	6/12/57	11/12/57	I
D-1	Greenwich	NJ 17	7/12/57	11/12/57	I
E-1	Bivalve	NJ 19	22/12/57	8/1/58	III MPN and SPC
B-2	Robbins Bros.	NJ 6	20/12/57	8/1/58	III MPN and SPC
D-2*	Greenwich	NJ 17	3/1/58	8/1/58	I
A-2	Ballard	Va 207	2/1	8/1	I
E-2	Bivalve	NJ 19	17/1	23/1	II SPC
F-1*	Roaring Point	Md 296	-	23/1	II MPN and SPC
A-3	Ballard	Va 207	16/1	23/1	I
D-3*	Greenwich	NJ 17	17/1	23/1	II MPN and SPC
G-1	Harris Co.	Md 264	28/1	4/2	I
E-3	Bivalve	NJ 19	24/1	4/2	II SPC
B-3	Robbins Bros.	NJ 6	28/1	4/2	III MPN and SPC
A-4	Ballard	Va 207	30/1	4/2	I
B-4	Robbins Bros.	NJ 6	15/2	19/2	III SPC
E-4	Bivalve	NJ 19	14/2	19/2	I
D-4*	Greenwich	NJ 17	14/2	19/2	I
B-5*	Robbins Bros.	NJ 6	1/3	5/3	III MPN and SPC
A-5*	Ballard	Va 207	20/2	5/3	II SPC
B-6	Robbins Bros.	NJ 6	1/3	5/3	II MPN and SPC
D-5	Greenwich	NJ 17	26/2	5/3	I
B-7	Robbins Bros.	NJ 6	3/3	7/3	I
B-8	Robbins Bros.	NJ 6	7/3	11/3	I
B-9	Robbins Bros.	NJ 6	24/3	1/4	III SPC
B-10	Robbins Bros.	NJ 6	28/3	2/4	I
B-11	Robbins Bros.	NJ 6	7/4	10/4	II MPN and SPC
B-12	Robbins Bros.	NJ 6	11/4	16/4	I

*Select Oysters

Oyster Samples having Positive Eijkmans which did not confirm as E. coli, Type I

Sample No.	No. of Positive Tubes								
	Coliform	Eijkman	E. coli	C_2	I_1	I_2	A_1	A_2	Other
3	9	3	2				1		
9	15	2	0						2 (NR + VP$_2$) ⓘ
26*	15	5	0		1		3		1 (NR + VP$_2$)
27*	9	4	3						1 (NR + VP$_2$)
29*	10	6	0			1	4		1 (A. cloacae)

* Eijkman incubation temperature not constant.

ⓘ Produced only small amount of gas and only on 48 hrs. incubation at 44.6°C.

APPENDIX C

OFFICE OF THE PRESIDENT

Board of Health

City of Chicago 10

Herman N. Bundesen, M. D.
President

August 15, 1958

Mr. Eugene Jensen
Chief, Shellfish Sanitation Program
Division of Sanitary Engineering Services
United States Public Health Service
U. S. Department of Health, Education and Welfare
Washington, D. C.

Dear Mr. Jensen:

Enclosed herewith is a "Report of Analysis of Samples of Shellfish Collected by the Division of Environmental Sanitation Chicago Board of Health ...."

This report is being forwarded to you in order that this information might be available for the forthcoming shellfish sanitation conference to be held in Washington on August 27-28.

The report indicates that of a total of 360 specimens collected and analyzed at the Board of Health laboratories, 51 were found to fall into class 2 (Acceptable on Condition) and 23 samples fell into class 3 (Rejectable). Investigation carried out by ourselves in the Chicago marketing area and the results of investigations carried out in shipping areas and forwarded to us would seem to indicate that, in addition to rigid sanitary control of beds and plants, there is a need for more adequate regulation of the packaging and transportation of shellfish.

With regard to the transportation of oysters from producing points to inland marketing areas, I would like to suggest that a study be made of possible variations from legal temperatures during the hours or days that shucked oysters are in transit from sea-board plants to inland marketing areas.

This can be accomplished through the inclusion of small recording thermometers with shipments. Data so obtained would prove of inestimable value in tracing possible causes for high coliform MPN's which would place shipments in the rejectable classification.

I wish to take this opportunity to thank you and the members of the staffs of the various State boards of health for cooperating so magnificently in maintaining public health standards in the shellfish industry.

Yours very truly,

/s/

Edward F. King,
Chief Sanitary Officer

REPORT OF ANALYSIS OF SAMPLES OF SHELLFISH COLLECTED BY THE DIVISION OF ENVIRONMENTAL SANITATION CHICAGO BOARD OF HEALTH, AND ANALYZED BY THE DIVISION OF LABORATORIES, CHICAGO BOARD OF HEALTH, FOR THE PERIOD FROM SEPTEMBER 1957 TO MAY 1958

COLLECTION DATE	FOOD SAMPLE NUMBER		SAMPLE	COLIFORM MPN PER 100 ML.
	Shipper's Name: Herman Thompson Gransonville MD #176			
9-23-57	40979	....	Shucked Oyster	...** 240,000
9-30-57	42538	....	" "	....* 92,000
10-14-57	43586	....	" "	....* 24,000
10-21-57	43604	....	" "	 3,300
10-23-57	43608	....	" "	 3,300
10-28-57	44458	....	" "	....* 18,000
10-30-57	44461	....	" "	....* 54,000
11-4-57	44471	....	" "	....* 54,000
11-7-57	44476	....	" "	...** 240,000
11-12-57	44483	....	" "	....* 28,000
11-18-57	44500	....	" "	...** 240,000
11-25-57	46137	....	" "	 2,700
12-2-57	46148	....	" "	 260
12-4-57	46152	....	" "	 2,700
12-9-57	46159	....	" "	...** 240,000
12-12-57	47126	....	" "	 7,900
12-16-57	47131	....	" "	 1,400
12-19-57	47136	....	" "	 790
12-30-57	47141	....	" "	 1,700
1-2-58	47145	....	" "	....* 35,000
1-6-58	47150	....	" "	 9,200
1-6-58	47151	....	" "	 7,900
1-8-58	47156	....	" "	.... 230 (Less than)
1-14-58	47166	....	" "	 780
1-20-58	27040	....	" "	....* 24,000
1-29-58	1766	....	" "	 2,200
2-3-58	72567	....	" "	.... 230 (Less than)
2-6-58	2767	....	" "	 1,700
2-10-58	2773	....	" "	 1,100
2-17-58	2783	....	" "	 2,600
3-4-58	3249	....	" "	.... 230 (Less than)

* Acceptable on condition - 8
** Rejectable - 4

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper - Herman Thompson (Continued)			
3-10-58	3922	Shucked Oyster	230 (Less than)
3-12-58	3927	" "	230 (Less than)
3-17-58	5535	" "	* 18,000
3-24-58	5545	" "	230 (Less than)
3-26-58	5553	" "	1,700
3-31-58	5273	" "	* 92,000
4-7-58	5282	" "	230 (Less than)
Shipper's Name: W. A. Thomas & Son Gransonville MD #159			
9-30-57	42539	" "	7,000
10-14-57	43585	" "	* 24,000
10-17-57	43592	" "	480
10-17-57	43593	" "	450
10-21-57	43603	" "	230
10-23-57	43607	" "	* 28,000
10-28-57	44457	" "	2,700
10-30-57	44462	" "	3,400
11-7-57	44475	" "	** 240,000
11-4-57	44470	" "	** 160,000
11-12-57	44482	" "	2,300
11-21-57	46130	" "	** 160,000
11-18-57	44490	" "	2,300
11-25-57	46136	" "	4,900
12-2-57	46147	" "	1,700
12-4-57	46151	" "	* 54,000
12-9-57	46158	" "	2,200
12-12-57	47127	" "	930
12-16-57	47130	" "	9,500
12-19-57	47135	" "	* 92,000
12-30-57	47140	" "	4,900
1-2-58	47144	" "	260
1-8-58	47155	" "	1,700
1-14-58	47165	" "	** 240,000
1-20-58	27039	" "	230 (Less than)
1-22-58	1752	" "	230 (Less than)
1-27-58	1765	" "	* 18,000
2-3-58	72566	" "	230 (Less than)
2-6-58	2768	" "	2,200
2-10-58	2772	" "	** 240,000

*Acceptable on condition - 7
**Rejectable - 5

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE		COLIFORM MPN PER 100 ML.
Shipper - W. A. Thomas & Son				
2-17-58	2782	Shucked Oyster		2,600
3-4-58	3250	"	"	2,200
3-10-58	3921	"	"	230 (Less than)
3-12-58	3928	"	"	490
3-17-58	5534	"	"	230 (Less than)
3-31-58	5272	"	"	230 (Less than)
3-24-58	5544	"	"	680
4-7-58	5281	"	"	230 (Less than)
Shipper's Name: J. H. Miles & Son Norfork Va. #214				
12-19-57	47134	Shucked Oyster		410
2-25-58	3238	"	"	330
2-25-58	3239	"	"	230 (Less than)
2-27-58	3241	"	"	4,000
2-27-58	3242	"	"	230 (Less than)
Shipper's Name: Bay Food Produce Co. Baltimore MD #12				
9-26-57	42527	Shucked Oyster		3,300
9-26-57	42528	"	"	11,000
10-9-57	42552	"	"	54,000*
10-9-57	42551	"	"	230
10-23-57	43606	"	"	7,000
10-31-57	44464	"	"	160,000**
10-31-57	44465	"	"	690
11-21-57	46132	"	"	1,300
11-21-57	46131	"	"	3,400
1-22-58	1753	"	"	490
3-6-58	3913	"	"	390
3-6-58	3914	"	"	780
3-14-58	5290	"	"	35,000*
3-16-58	8026	"	"	230 (Less than)
3-16-58	8027	"	"	390
3-19-58	5539	"	"	3,400
3-19-58	5540	"	"	5,400

*Acceptable on condition 2
**Rejectable - 1

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper - Bay Food Produce Co. (Continued)			
4-9-58	5285	Shucked Oysters	1,100
4-9-58	5286	" "	230 (Less than)
4-21-58	8028	" "	230 (Less than)
4-21-58	8029	" "	230 (Less than)
4-23-58	8030	" "	230 (Less than)
4-23-58	8031	" "	7,900
Shipper's Name: Northern Oyster Co. Greenport L.I. N. Y. #24			
9-11-57	40968	Shell Oysters	490
Shipper's Name: Greenport Oyster Co. Greenport, N.Y. #95			
9-16-57	40973	Shell Oysters	1,700
Shipper's Name: L. R. Larson, Inc. Crisfield, MD			
10-1-57	42540	Shell Oysters	* 35,000
Shipper's Name: Crisfield Pkg. Co. Baltimore, MD #199			
10-10-57	42556	Shell Oysters	3,300
11-8-57	44478	Shucked Oysters	2,100
11-20-57	46128	" "	2,800
12-11-57	46164	" "	* 240,000
9-9-58	47159	" "	4,300
9-23-58	1754	" "	1,700
9-31-58	1769	" "	* 24,000
2-7-58	2770	" "	4,600
2-14-58	2779	" "	9,500
2-19-58	3232	" "	1,400
3-27-58	5261	" "	* 54,000
4-2-58	5278	" "	690
4-8-58	5284	" "	230 (Less than)

* Acceptable on condition - 3
** Rejectable - 1

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
		Shipper's Name: Wariwick & Ashburn Wiems, VA #133	
3-6-58	3912	Shucked Oysters	800
		Shipper's Name: G. T. Elliah, Inc. Hampton, VA. #59	
3-12-58	3925	Shucked Oysters	8,100
		Shipper's Name: Greenwich Oyster Co. Greenwich, N. J. #17	
10-25-57	44453	Shucked Oysters	1,300
		Shipper's Name: Shelter Island Oyster Co. Greenport, N. Y. #133	
9-9-57	33814	Shell Oysters	13,000
2-19-58	3233	" "	230 (Less than)
		Shipper's Name: Harry C. Taylor Chincoteague, Va. #87	
9-18-57	40975	Shucked Oysters *	54,000
		Shipper's Name: Edward M. Plitt & Sons Oyster, Va. #75	
1-23-58	1755	Shucked Oysters	13,000
2-6-58	2766	" "	1,100
3-12-58	3926	" "	330
3-20-58	5542	" "	230 (Less than
		Shipper's Name: O. E. Wentworth & Co. Baltimore, MD #45	
2-13-58	2778	Shucked Oysters	230 (Less than)

* Acceptable on condition - 1
** Rejectable - 0

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
	Shipper's Name: Peterson Pkg. Co. Norris, N. J. #16		
10-3-57	42544	Shucked Oysters	230 (Less than)
10-10-57	42557	Shucked Oysters	330
10-15-57	43589	" "	1,100
10-30-57	44463	" "	13,000
11-8-57	44479	" "	780
11-22-57	46133	" "	* 240,000
11-29-57	46144	" "	2,700
12-5-57	46153	" "	230 (Less than)
1-8-58	47157	" "	6,200
1-17-58	47170	" "	3,100
1-31-58	1770	" "	230
	Shipper's Name: Greenwich Oyster Co. Greenwich, N. J. #17		
3-27-58	5262	Shucked Oysters	* 18,000
4-2-58	5279	" "	* 35,000
	Shipper's Name: West Oyster Co. Oysger, Va. #11		
4-9-58	5287	Shucked Oysters	330
	Shipper's Name: Allen Kirkpatrick Rehaborh, Del. #36		
9-9-57	33813	Shucked Oysters	7,900
	Shipper's Name: Tallmadge Bros. So. Norwalk, Conn. #10		
11-6-57	44472	Shucked Oysters	2,300

* Acceptable on condition - 2
** Rejectable - 1

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
	Shipper's Name:	Blue Point Co. West Sayville, N. Y. #24	
2-17-58	2781	Shell Oysters	230 (Less than)
	Shipper's Name:	Blue Print Co. West Sayville, N. Y. #7	
3-13-58	5527	Shell Oysters	230 (Less than)
	Shipper's Name:	Shreves Bros. Chincoteague, Va #312	
3-18-58	5533	Shucked Oysters ..... *	35,000
	Shipper's Name:	Wentworth Co. Baltimore, MD. #145	
4-8-58	5283	Shucked Oysters	7,000
	Shipper's Name:	Woodfield Fish, Oyster Co. Galleaville, MD. #81	
3-14-58	5288	Shucked Oysters	490
3-26-58	5551	" "	230 (Less than)
	Shipper's Name:	Savage & Meers Chincoteague, Va. #235	
10-24-57.....	44451	Shucked Oysters	230 (Less than)
10-28-57.....	44456	Shucked Oysters ..... *	17,000
	Shipper's Name:	Savage & Meers Chincoteague, Va. #151	
9-16-57.....	40971	Shucked Oysters ... A*	240,000
9-23-57	40978	" "	11,000
9-30-57	42536	" "	2,200
10-4-57	42545	" " ..... *	35,000

Class II

* Acceptable on condition - 3
** Rejectable - 1

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Savage & Meers (Continued)			
10-7-57	42547	Shucked Oysters	4,900
10-14-57	43583	" "	4,900
11-4-57	44469	" "	14,000
11-12-57	44481	" "	3,200
11-18-57	44498	" "	4,700
11-25-57	46135	" "	1,700
12-2-57	46146	" "	5,400
12-9-57	46157	" "	3,300
12-16-57	47129	" "	7,900
12-30-57	47139	" "	470
1-2-58	47142	" "	4,900
1-13-58	47163	" "	230 (Less than)
1-20-58	27038	" "	330
1-27-58	1761	" "	330
1-30-58	1767	" "	230
2-4-58	2762	" "	3,400
2-11-58	2774	" "	490
2-18-58	3229	" "	1,100
2-28-58	3243	" "	230 (Less than)
3-3-58	3248	" "	4,600
3-10-58	3919	" "	2,700
4-1-58	5274	" "	230 (Less than)
	Shipper's Name:	Blue Point Co., Inc. West Sayville, N.Y. #7	
9-10-57	33815	Shell Oysters	1,100
11-6-57	44473	Shucked Oysters	1,400
12-17-57	47133	" "	330
1-2-58	47143	" "	260
1-30-58	1768	" "	1,100
	Shipper's Name:	Tallmadge Bros. So. Norwalk, Conn. #10	
11-13-57	44484	Shucked Oysters	2,700
	Shipper's Name:	Bevalve Pkg. Co. Bevalve, N.J. #19	
12-5-57	46154	Shucked Oysters	2,600

Acceptable on condition - 0
Rejectable - 0

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper's Name: Robbins Bros. Port Norris, N. J. #6			
9-30-57	42537	Shucked Oysters	480
10-7-57	42548	" "	450
10-18-57	43594	" "	230 (Less than)
10-24-57	44452	" "	1,300
11-1-57	44466	" "	1,300
11-21-57	46129	" "	*240,000
11-27-57	46141	" "	1,100
1-10-58	47160	" "	* 54,000
1-24-58	1756	" "	4,900
2-4-58	2763	" "	* 35,000
2-11-58	2775	" "	*240,000
2-18-58	3230	" "	14,000
2-25-58	3237	" "	*160,000
3-7-58	3915	" "	2,700
3-10-58	3920	" "	540
3-18-58	5536	" "	1,700
Shipper's Name: Blue Point Co. West Sayville L.I. N.Y. #19			
1-17-58	47169	Shucked Oysters	230 (Less than
Shipper's Name: Blue Point Co. West Sayville, N.Y. #24			
3-26-58	5552	Shell Oysters	230 (Less than)
Shipper's Name: Ralph Watson Chincoteague, Va. #283			
9-24-57	40980	Shucked Oysters	**160,000
9-18-57	40974	" "	* 28,000
10-1-57	42542	" "	1,100
10-8-57	42549	" "	3,300
10-16-57	43590	" "	**160,000
10-29-57	44460	" "	2,300
11-5-57	44474	" "	**240,000
11-19-57	46125	" "	930
11-26-57	46140	" "	2,600
12-3-57	46150	" "	13,000

* Acceptable on condition - 3
** Rejectable - 6

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper - Ralph Watson (Continued)			
1-3-58	47147	Shucked Oysters	450
1-7-58	47152	" "	490
1-16-58	47168	" "	* 21,000
1-21-58	1748	" "	240
1-29-58	1764	" "	2,400
2-5-58	2765	" "	* 28,000
2-13-58	2777	" "	4,500
2-18-58	3231	" "	1,400
2-28-58	3245	" "	230 (Less than)
3-5-58	3911	" "	490
3-11-58	3923	" "	4,500
3-14-58	5289	" "	* 54,000
3-20-58	5541	" "	370
3-25-58	5549	" "	3,200
4-1-58	5275	" "	1,700
4-7-58	5280	" "	1,300
Shipper's Name: Allen Kirkpatrick Rehoboth, Del. #36			
10-9-57	42554	Shucked Oysters	2,300
10-9-57	42553	" "	2,300
10-22-57	43762	" "	2,600
10-29-57	44821	" "	4,900
11-5-57	44894	" "	2,300
11-12-57	44905	" "	* 35,000
11-19-57	44921	" "	** 240,000
12-4-57	45901	" "	1,300
12-10-57	46885	" "	2,000
12-17-57	47625	" "	610
1-7-58	379	" "	* 92,000
1-14-58	391	" "	2,100
2-4-58	1915	" "	5,400
2-11-58	1926	" "	1,400
2-18-58	1939	" "	4,900
2-26-58	2522	" "	230 (Less than)
3-4-58	3157	" "	230 (Less than)
3-18-58	4493	" "	490
3-25-58	4510	" "	1,100
4-1-58	4526	" "	3,400
4-8-58	5204	" "	4,700
4-15- 8	5223	" "	490
4-22-58	5245	" "	3,300

* Acceptable on condition - 5
** Rejectable - 1

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper's Name: Somerset Seafood Co. Deals Island, MD #304			
9-12-57	40970	Shucked Oysters	20 (Less than)
9-26-57	42526	" "	54,000*
9-27-57	42529	" "	4,900
10-3-57	42543	" "	17,000*
10-10-57	42555	" "	7,900
10-14-57	43584	" "	35,000*
10-15-57	43587	" "	13,000
10-21-57	43601	" "	4,600
10-23-57	43605	" "	17,000*
10-28-57	44455	" "	3,400
11-4-57	44468	" "	92,000*
11-12-57	44480	" "	5,400
11-18-57	44497	" "	5,400
11-20-57	46126	" "	22,000
11-25-57	46134	" "	3,200
12-2-57	46145	" "	1,100
12-9-57	46156	" "	1,700
12-17-57	47132	" "	330
12-20-57	46165	" "	92,000*
1-6-58	47148	" "	11,000
1-13-58	47162	" "	1,100
1-20-58	27037	" "	7,000
1-27-58	1760	" "	2,700
2-4-58	1771	" "	11,000
2-10-58	2771	" "	3,300
2-20-58	2785	" "	3,100
2-20-58	2784	" "	1,300
2-20-58	2786	" "	4,600
2-24-58	3234	" "	1,700
2-24-58	3235	" "	54,000*
2-24-58	3236	" "	470
3-3-58	3247	" "	2,800
3-3-58	3246	" "	9,500
3-10-58	3917	" "	1,700
3-10-58	3918	" "	3,200
3-17-58	5531	" "	7,900
3-17-58	5532	" "	17,000*
3-24-58	5547	" "	13,000
3-24-58	5546	" "	540
3-31-58	5271	" "	2,200
3-31-58	5270	" "	1,400
4-2-58	5277	" "	14,000

* Acceptable on condition - 8
** Rejectable - 0

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper's Name: Greenwich Oyster Co. Greenwich, N. J. #17			
9-24-57	40981	Shucked Oysters	1,300
4-1-58	5276	" "	* 35,000
Shipper's Name: Port Norris Oyster Co. Port Norris, N. J. 1			
10-1-57	42541	Shucked Oysters	11,000
10-8-57	42550	" "	230 (Less than)
10-16-57	43591	" "	11,000
10-25-57	44454	" "	* 35,000
10-29-57	44459	" "	* 17,000
11-7-57	44477	" "	* 54,000
11-14-57	44489	" "	* 17,000
11-19-57	46124	" "	* 92,000
12-3-57	46149	" "	13,000
12-10-57	46160	" "	1,100
12-13-57	47128	" "	3,900
12-17-57	47134	" "	1,100
1-7-58	47154	" "	* 160,000
1-14-58	47164	" "	2,800
1-22-58	1751	" "	230 (Less than)
1-28-58	1763	" "	9,200
2-5-58	2764	" "	230
2-11-58	2776	" "	690
2-28-58	3244	" "	230 (Less than)
3-4-58	3910	" "	4,900
3-11-58	3924	" "	230 (Less than)
3-18-58	5537	" "	** 160,000
3-25-58	5548	" "	1,300
3-28-58	5264	" "	2,700
Shipper's Name: George M. Stell, Inc. 228 Front St., N. Y. #2			
9-26-57	42525	Shell Oysters	230 (Less than
9-26-57	42524	" "	780
9-26-57	42523	" "	1,700
9-27-57	42530	" "	230 (Less than)
10-4-57	42546	" "	230 (Less than)
10-18-57	43595	" "	2,700

* Acceptable on condition - 6

** Rejectable - 2

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper - George M. Stell, Inc. (Continued)			
11-27-57	46142	Shell Oysters	230
1-3-58	47146	" "	620
1-10-58	47161	" "	230 (Less than)
1-17-58	47171	" "	230 (Less than)
1-24-58	1757	" "	230 (Less than)
2-7-58	2769	" "	230 (Less than)
2-14-58	2780	" "	230 (Less than)
3-7-58	3916	" "	230 (Less than)
3-13-58	5529	" "	230 (Less than)
3-21-58	5543	" "	330
3-28-58	5263	" "	230 (Less than)
Shipper's Name: Royal Molpique Summerside P.E.I. Ltd. Canada 161			
9-25-57	42521	Shell Oysters	* 54,000
11-13-57	44485	" "	330
11-27-57	46143	" "	230 (Less than)
Shipper's Name: Blue Point Co., Inc. West Sayville, L.I. N.Y. #24			
11-13-58	44486	Shell Oysters	330
Shipper's Name: Charles Newbert & Co. Baltimore, MD. #39			
11-26-57	46139	Shucked Oysters	2,600
12-10-57	46162	" "	* 24,000
1-21-58	1750	" "	* 25,000
Shipper's Name: S. L. Coward Lottsburg, Va. #96			
10-21-57	43602	Shucked Oysters	** 160,000
Shipper's Name: Harry Collins Franklin City, Va. #246			
10-15-57	43588	Shucked Oysters	14,000

* Acceptable on condition - 3
** Rejectable - 1

COLLECTION DATE	FOOD SAMPLE NUMBER	SAMPLE	COLIFORM MPN PER 100 ML.
Shipper - Harry Collins (Continued)			
11-19-57	46123	Shucked Oysters	240 (Less than)
12-6-57	46155	" "	3,300
11-26-57	46138	" "	330
12-26-57	47138	" "	1,200
1-7-58	47153	" "	230 (Less than)
1-28-58	1762	" "	230 (Less than)
3-4-58	3909	" "	230 (Less than)
3-14-58	5530	" "	230 (Less than)
	Shipper's Name: Harry Collins Franklin City, Va. #283		
1-21-58	1749	Shucked Oysters	230 (Less than)
	Shipper's Name: Ballard Fish & Oyster Co. Norfolk, Va. #207		
2-27-58	3240	Shucked Oysters	340
3-25-58	5550	" "	2,600

Acceptable on condition - 0
Rejectable - 0

SUMMARY:
Total Samples Collected - 360
*Acceptable on condition - 51
**Rejectable - 23

August 12, 1958

APPENDIX D
BACTERIOLOGICAL QUALITY OF MARKET OYSTERS IN LOUISIANA

By

Dr. George H. Hauser, Director
Division of Laboratories
Louisiana State Department of Health

The State of Louisiana has a serious responsibility in the field of shellfish sanitation. The vast areas in the lower Mississippi Delta area seem to be ideally suited for the propagation of oysters. Louisiana has long recognized the importance of these natural resources and in the field of public health as well as in conservation there has been for many years a close system of control. The Louisiana State Health Department, in the Division of Laboratories and the Division of Food and Drugs has exercised sanitary control of the harvesting and packing of shellfish for many years. In cooperation with the Division of Sanitary Engineering the Division of Laboratories and the Division of Food and Drugs conducted a comprehensive sanitary survey of the shellfish growing areas in the State which involved the collection and examination of many thousands of samples of water and oysters throughout a period of more than five years.

The survey demonstrated that with very little exception the shellfish growing areas in the State of Louisiana are quite remote from pollution and yet the bacteriological quality of shell and shucked oysters as found in the shucking houses and as measured by the current standards would be classified as poor to bad. We have long recognized that in our areas the bacteriological indices developed for shellfish as harvested and packed in the more northern sections of the country were not entirely satisfactory and for this reason we have applied for many years a test for the presence or absence of *E. coli*. Upon the development of more advanced procedures for the detection and enumeration of these organisms we anxiously applied and evaluated this test in our State.

The results of our bacteriological examinations of waters and shellfish as harvested and as shucked and packed in Louisiana shucking houses from 1951 have been submitted to the Public Health Service for comment and review. A summary of the results of examinations conducted throughout this seven year period has shown the results listed in Table 1.

TABLE 1

MAXIMUM, MINIMUM AND MEAN COLIFORM MPN'S ON SHELL AND SHUCKED OYSTERS

	Coliform MPN		
	Shell Oysters(1)	Shucking Plant(2)	
	As Dug	Shell	Packed
Maximum	1,700	160,000+	160,000+
Minimum	<23	<23	20
Mean	110	5,400	16,000

(1) Forty-nine samples as harvested during the course of sanitary surveys.

(2) Ninety-nine samples collected in the routine bacteriological sampling program of oyster houses 1950-1956

The data clearly shows the problem. The average coliform MPN of samples collected from representative oyster harvesting areas during the course of sanitary surveys was 110 and the highest coliform MPN on shell oysters taken during the course of these surveys was 1,700. Only three of 49 samples were in excess of 1,000. Samples of shell oysters taken at the shellfish shucking houses during the course of routine inspections and examinations conducted from 1950 to 1958 show an average coliform MPN of shell stock of 5,400 and approximately one-fourth of these oysters show coliform MPN's of more than 16,000.

The sanitary conditions of transportation of these shell oysters from the harvesting area to the shucking house is by no means unsatisfactory. These increases in coliform bacteria obviously are the result of multiplication which occurs with time at the temperatures prevailing in this area. Since the shellfish come from areas of good to excellent sanitary quality the significance of these multiplications as they pertain to a potential public health danger is in my opinion very low. We have long recognized that it would be desirable to be able to produce a product of lower bacterial content. Many of the oyster packers also realize this and exercise as good practices as can be done within the limits of practicality. During the warmer months the shell oysters on arrival at the packing house are refrigerated and remain so until immediately before shucking.

As indicated previously we collaborated with the Public Health Service, Shellfish Sanitation Laboratory in studies to determine the exact cause of these high coliform values by conducting a special study which involved the collection and examination of traced lots of oysters from the time of arrival of the harvesting boat until the shucked product was finally packed. In the examinations during the past oyster harvesting season the patterns of changes in coliform MPN and Plate Count was not materially different than those which we had previously observed during the course of our routine inspections and examinations. In these studies we applied the test for E. coli as developed for the study underway in the North. The relative value of these two indicator organisms I believe is quite well illustrated in the following Table 2.

TABLE 2

BACTERIOLOGICAL RESULTS ON SHELL AND SHUCKED OYSTERS - SPECIAL STUDY
HARVESTING SEASON 1957-1958

	Shell Oysters		Shucked Oysters	
	As Landed	At Plant	As Shucked	As Packed
		Coliform MPN		
Maximum	54,000	160,000	160,000	92,000
Minimum	68	40	130	790
Median	5,400	5,400	4,300	4,300
		E. Coli Completed Test MPN		
Maximum	310	230	1,300	330
Minimum	<18	<18	<18	<18
Median	20	20	20	45
		Standard Plate Count		
Maximum	11,000	49,000	48,000	150,000
Minimum	840	400	1,800	3,300
Median	3,100	6,900	33,000	22,000

This data shows the relative value of the coliform group and E. coli as indices of pollution. Comparison of the average coliform MPN of 110 in oysters as dug with those at later stages in handling and packing shows multiplication at points where there is little possibility of contamination. Although we have no figures for E. coli MPN of oysters as dug they certainly are still low on arrival at the shucking house and therefore because of the little change in the interim between harvesting and shucking should serve as a better index of pollution. The gradual increase in Plate Counts demonstrate the value of this test in evaluating handling practices.

We are hopeful that the group will give serious consideration to the use of the E. coli test as an index of sanitary quality of shellfish. We feel that particularly in those areas where either naturally occurring coliforms or those which come into the product in spite of good handling practices, are inordinately high, there is a serious need for another indicator organism. The Plate Count has its place as an index of mishandling or poor storage practices but in the southern areas the coliform group of organisms leaves much to be desired as an index of pollution.

APPENDIX E

F.D.A. STANDARDS OF IDENTITY FOR SHELLFISH

by

Shelbey T. Grey, Director
Bureau of Program Planning and Appraisal
Food and Drug Administration
Department of Health, Education, and Welfare

Today, in our high-speed civilization, there are few problems in the transmission of news and other information. But real problems exist in the communication of ideas between groups in industry and government, looking toward better understanding and cooperation. The workshop meeting is a fruitful medium for such communication. I am therefore happy to be with you today so that we can consider those common areas embraced by the Food and Drug Administration's shellfish standards and their enforcement and your shellfish sanitation activities.

Before I go into a technical discussion of these areas however, I would like to call to your attention a sinister plot against the oyster per se. There is a story going around that when God made the oyster, He guaranteed him absolute economic and social security. He built the oyster a house, a shell to protect him from his enemies. When hungry, the oyster simply opens his shell and food rushes in to him.

But when God made the eagle, He said, "The blue sky is the limit. Go build your own house," and the eagle built on the highest mountain crag, where storms threaten him every day. For food, he flies through miles of rain and snow and wind.

The eagle, and not the oyster, is the symbol of America.

So, gentlemen, the oyster is being branded as un-American--I daresay, if those who initiated this comparison knew more about oyster biology, they would have a greater respect for the oyster.

The Federal Food, Drug, and Cosmetic Act of 1938 gave us the legal authority to promulgate standards of identity and quality for foods to promote honesty and fair dealing in the interest of consumers. We promptly began experiments to secure data on shucked oysters, looking toward early establishment of standards. We had great hopes that a standard would help us control the adulteration of shucked oysters with water, that is, excessive amounts of water, for we recognized that shucked oysters had to be washed to produce a clean product, acceptable to the consumer.

These experiments, from 1940 to 1945, attempted to arrive at washing procedures adequate to produce a clean product yet with a minimum of water absorption and loss of solids in the final product. If we allowed washing in fresh water, and that was the usual industry practice, it was necessary to set limits for the amount of time shucked oysters could be in contact with water. We knew from the classical experiments of the late Dr. Albert Hunter, Chief, Division of Microbiology, FDA, and others, that washing in salt water, above 0.75 per cent salt, did not result in water absorption and loss of solids. Dr. Hunter, in U.S.D.A. Technical Bulletin 64, published in 1928, stated:

"To sum up the present knowledge of the bacteriology involved in the washing process; hosing or spraying the oysters with water on a skimmer will not reduce the bacterial contamination due to shucking and handling, but washing in a blower in a weak brine from three to five minutes will reduce the bacterial content to about that of the oysters before shucking, without undue bloating."

I do not know if these conclusions were still valid at the time we were doing our standards experiments in the early 1940's, since we did not run any bacteriological tests. Here was an area - the bacteriology involved in the washing process - in which you, as shellfish sanitarians, were vitally interested. Yet, for some reason, communications broke down between our two groups.

The oyster standard of identity has now been in effect for eleven years. In its enforcement we have observed considerable variation in the type and design of equipment used in washing and draining of oysters. In some cases the equipment is so designed that thorough drainage, as required by the standard, is hard to achieve. We note with interest that your agenda covers the topic "Construction Standards for Equipment Used by the Shellfish Industry." Here is an area of common concern.

Opportunity, once lost, it is frequently stated, does not knock again. Fortunately, this is not always true. And sometimes adversity creates an opportunity. This has been our recent experience as a result of an adverse decision of Federal Judge Sterling Hutcheson, denying our request for an injunction against an oyster packer charged with shipping adulterated oysters. In his decision, Judge Hutcheson pointed out that the scientific evidence presented by the Food and Drug Administration to support the charge of adulteration of oysters with water, conflicted with certain statements in the Findings of Fact in the original oyster standards hearings held in 1945 and 1946. Judge Hutcheson suggested that the standards should be amended to harmonize the Findings of Fact with the scientific evidence.

The $64,000 question before the Food and Drug Administration, the oyster industry, and the U.S. Fish and Wildlife Service was, "What Should Be Done About the Oyster Standards?" Fortunately, each group did not, like Achilles, retire to their tents and sulk. Thanks to the statesmanlike approach of Mr. David Wallace, Secretary of the Oyster Institute of North America, arrangements were made for a panel discussion on this question during the 50th annual convention of the Institute on July 22, 1958 in Baltimore and the Food and Drug Administration was invited to participate. I had the honor and pleasure of speaking for the Food and Drug Administration.

I will not attempt to recapitulate the substance of my talk. Among other things, it states our present enforcement policy. Since you may be interested, I have copies of the talk available for your leisurely reading. I do wish, though, with your indulgence, to mention a positive achievement which resulted from the panel discussion. We proposed that the oyster industry, represented by the Oyster Institute of North America, the U.S. Fish and Wildlife Service, and the Food and Drug Administration undertake cooperative research as the best means for arriving at the scientific facts at issue. At the conclusion of the convention, the industry voted to join in such a research program. The Fish and Wildlife Service likewise agreed to join in the program.

The Planning Committee for the cooperative research program representing the three groups has prepared a Prospectus. You have all received copies. A while back I mentioned that opportunity may knock twice. The Planning Committee earnestly hopes that you, as shellfish regulatory officials, will take this opportunity to help us, and yourselves, on this cooperative research program. You can help in the following ways:

1. Review the tentative list of Proposed Oyster Research Projects in Appendix A.

2. Give consideration to additional projects which you believe should be included in the program.

3. If you have any experimental data, either published or unpublished, which bears on the identity or quality of shucked oysters, consider making it available to the cooperative research team.

4. Communicate your ideas and your data to the Planning Committee. They will be most welcome. You may address such communications to Mr. David Wallace, Mr. Charles Butler of the U.S. Fish and Wildlife Service, or myself.

You will note that we estimate that our research program will take two years. We hope that there will never be a time limit to the opportunities for communication between our groups. For, as the late E. T. Meredith*, successful publisher, once said, "If one hundred men each tell me something they have learned as the result of ten years experience, I am in a position to profit by their combined wisdom; that is, by one thousand years of experience."

*Mr. Meredith was publisher of the Farmer's Tribune and Successful Farming. He was a director of the Chicago Federal Reserve Bank and Secretary of Agriculture in the cabinet of President Woodrow Wilson.

APPENDIX F
ADMINISTRATION OF PROGRAM OF CERTIFICATION OF SHELLFISH SHIPPERS - THE INDUSTRY VIEWPOINT

by

David H. Wallace, Director
Oyster Institute of North America

Certification of interstate shellfish shippers and the successful administration of the program by the State Health Departments and the U. S. Public Health Service has been one of the most important contributions to the welfare of the shellfisheries in the last thirty years. Despite misunderstandings which have arisen from time to time between the three parties directly concerned, the program has progressed steadily, with a constant improvement in the market quality of the domestic product. Great improvements have taken place in the last 18 years which is the period in which I have had direct contact with the industry.

Implant conditions have so improved today that there is small liklihood of any illness resulting from consumption of oysters and other shellfish whether they be fresh, frozen, or processed. Shellfish still must be transported from the packing site to the dealer, who holds the product until distributed to the retailer. At this level a good shellfish product at the original shipping point may be allowed to deteriorate rendering it unsafe or unfit for food.

This marketing procedure is outlined to emphasize that production of our product entails several channels of trade, each of which has a considerable affect on sanitary quality. Conceivably a poor product can be offered the consumer which was packed using the best known sanitary criteria. The question has been asked by certain packers where their responsibility ends in the chain. The answer is that responsibility ends only with a satisfied consumer, even though packer controls over truckers, dealers, and retailers are practically non-existent. It is all very well to say that packers should use only reliable transportation facilities and ship to legitimate dealers. Even when these prerequisites are met, the product sometimes shows high bacterial count in the market. Possibly this should be explored to ascertain if safeguards can be developed which would bridge the gap between packer and market.

Administration of the cooperative program cannot be dealt with adequately here. It would require a detailed analysis and evaluation of the industry-state health agency relationship in every coastal state. The effectiveness of the coordination provided by the Public Health Service in each regional district and between the regions is another phase which cannot be examined thoroughly. Consequently, the industry viewpoint must deal in generalities rather than specifics. This usually ends only in frustration and disagreement, but it will be attempted anyway.

The major complaint of the industry about the federal and state activities in the program is the failure to attain uniformity either in the coordinating function or in the interpretation of enforcement requirements by the states. At the federal level this lack of uniformity is apparent between districts. One possible cause is inadequately trained personnel in some of the districts to carry out the functions necessary under the cooperative program.

This results in wide discrepancies between areas such as the North and Middle Atlantic and the Gulf, and indeed different interpretations of the Public Health manual itself. I am well aware of the difficulties in attaining uniformity. However, the discrepancies do exist and in our opinion should be corrected.

Another problem on the federal level has been the recurring expression to me by some packers that the revised manual is more stringent than had been agreed upon by industry in the formative conferences - and needlessly so. This complaint arises from the necessity of substantial outlays of cash to replace equipment and make plant alterations to conform to the recommendations prescribed in Part II of the Manual of Recommended Practice. When a man is injured in his pocket book, he is sorely hurt indeed. Packers, who have been shipping consistently a sanitary product in the Chesapeake area, feel that this fact alone is proof that some of the changes were unnecessary.

The third problem in the federal activity is that personnel in some districts apparently have assumed enforcement duties on a state level which was not contemplated under the industry-state-federal program. The industry has looked upon the federal shellfish sanitation field personnel as personal advisors and consultants on their sanitary problems. They welcomed their visits and inspections, since valuable comments and suggestions for improvement were forthcoming. This condition still exists in several districts and the plant operators respect the federal officer and enjoy their brief contact with him. Vast improvements have been attained by such an approach. We hope that the policy will be continued and extended to cover all Public Health Service shellfish field people.

The techniques and attitudes of a federal policeman results in resentment, devious resistance, and overall loss of respect for the role of the Public Health Service in the shellfish program. Unfortunately, in some sections the police authority attitude has been assumed, according to reports from packers.

This is regrettable since the industry generally appreciates the efforts of the Service in this activity, and takes their advice on sanitation problems seriously and with respect. This role of senior advisor - if you will allow me to create a non-existant office - is probably the strongest position in the entire program and one which we feel must be retained and guarded at all costs.

Discussion of industry-state relationships and problems is even broader than the industry-federal discussion I have just attempted. There are 21 coastal states producing shellfish. Each one has its own problems with its local industry. However, there is one common problem that exists everywhere - to enforce the same requirements sanitation-wise on every packer whether he operates in Massachusetts, Florida, Louisiana, or California.

Uniformity of enforcement is essential both within each individual state and between the several individual states. From my own personal observations in travelling from state to state and talking with industry and state sanitation personnel, present enforcement is far indeed from uniform. I have observed plants in some states within the jurisdiction of a single sanitarian where one packer would be ordered to make extensive changes and repairs. Another packer, whose plant appeared to be no better or cleaner, apparently was permitted to continue his operations without any modifications at all. I realize, of course, that things are not always as they seem, and maybe the "privileged" packer is really under a court order or faces other punitive action. The point I am trying to make is that every effort should be made to see that each packer, big or small, is required to do the same as his competitor. This applies to all sanitarians within a state, just as much as it does to a single one. When more than one sanitarian is involved, the possibilities for different interpretations are multiplied but the necessity for equal requirements is just as valid. This necessitates careful supervision of sanitarians, education of personnel as to the state requirements and interpretations of the manual, and constant rechecking of the numerous plants scattered over the area.

All of the activities of these people we have been discussing are within the boundaries of one state and subject to one control. How to attain uniformity between independent, sovereign states is a much more difficult matter. The authority of the Public Health Service cannot bring this about. The states themselves must take steps to accomplish this goal. How many states review together their enforcement activities on shellfish sanitation? Have New York and Connecticut attempted to coordinate their programs so that packers in Connecticut are required to do the same things as those in New York? What about New Jersey and Alabama?

Oysters are still sold to the consumer from all these states at a competitive price. I suspect that regional attempts are being made to bring about coordination in nearby states but what about widely separated ones such as Delaware and Texas?

There is another factor in the program which has disturbed the substantial members of the industry. A number of states impose more stringent regulations on those packers shipping interstate than for intrastate shipments. Actually this places the state health department in the position of saying "We are more interested in the health of the citizens of our neighboring state than our own." The industry feels strongly that all packers whether intrastate or interstate must conform to the same standards. If the intrastate requirements are all that are necessary to protect the public health, then interstate shippers are being harassed needlessly.

There is one final item which gives the shellfish industry grave concern. Oysters and other shellfish for food must be taken from pollution free waters as determined by sanitary water quality surveys, etc. This applies without exception, irrespective of the ultimate method of processing. This provision apparently is to protect consumers from polluted or filthy shellfish whether they are fresh, frozen, or canned.

Even though most states permit only certified shellfish to be brought into their territory from another state, most states are now permitting canned oysters and clams, and frozen clams to be imported from Japan, Hong Kong and other countries. Frozen oysters, undoubtedly will be next on the list. We feel that state authorities should halt these imports unless it can be shown conclusively that they came from pollution free waters and were packed in plants with sanitary standards equal to the minimum requirements of the Public Health Service Manual. Some states have advised that canned shellfish are not in the certification program so they cannot act on any canned oysters or clams. This appears to be merely dodging the issue.

Once again we have the legitimate oyster grower and packer penalized because he operates in such a way as to protect the public health of the consumer while importers can sell anything they can persuade the consumer to buy.

We urge this workshop to go on record opposing the importation of shellfish grown in polluted waters and packed in plants not meeting the minimum requirements as set forth in Part II of the Public Health Service manual. We urge further that this conference recommend to each state health department that appropriate steps be taken to protect the consumer by prohibiting any imports of shellfish in their state until such time as the Public Health Service can assure them that these shellfish were produced in pollution free waters and packed under sanitary conditions.

This action may be interpreted by some as being contrary to national policy. It has been contended that the objective examinations practiced by FDA at point of entry are sufficient safeguards. The facts are that imports of oysters are four times greater than they were 5 years ago. Thus far this year, a greater quantity has been imported than ever before in the same months. In Japan sanitation conditions are bad as can be seen here. We hope action will be taken to meet this problem.

APPENDIX G

PARALYTIC SHELLFISH SURVEY, MAINE - 1958

by

Mr. Phillip L. Goggins
Marine Resource Scientist
Department of Sea and Shore Fisheries
Boothbay Harbor, Maine

The only report in the literature on the incidence of paralytic shellfish poisoning in Maine is a U. S. Fish and Wildlife Service (1946) record of twenty-six cases in 1943.

Scattergood (1958) states, "It is my understanding that there have been no cases of mussel poisoning reported in Maine except during 1943. The 1943 report is not correct. There were no cases of mussel poisoning then. The twenty-five or twenty-six people became mildly ill, with headaches, slight nausea, etc., but there was no evidence that they had eaten any mussels. I believe there are no records of persons becoming ill from mussels except from eating them. Mr. Clyde Taylor and I were present at one of the two factories (Herrick and Allen of Brooklin) when the mild attacks occurred, for we were requested to look into the problem immediately. We bought a dozen chickens and fed them mussels, both canned and fresh, from the cannery's supply. Chickens are extremely sensitive to mussel toxin and die within a few minutes after ingesting a toxic mussel. The chickens were fed great quantities of mussels and survived, so there was no evidence of toxin.

As regards the illness, we suspect that it was faulty heating system that was the cause. The cannery was poorly ventilated, hot with much steam, and the boiler which used soft coal, was not separated by closed doors from the packing room. It was a poorly arranged set-up, and the cannery burned down several weeks later, probably because of the poor boiler installation.

There was another nearby cannery which also reported several people who did not feel well. We suspect, that these people were influenced by happenings at Herrick and Allen. Having been told that the Herrick and Allen workers were getting sick from handling mussels, some of the people in the second cannery decided that they too didn't feel quite as well as usual.

Occasionally a report of mass mortality in the sea bird population comes to our attention. In 1956 one was reported in the Eider duck on the island of Matinicus located fifteen miles off shore in Penobscot Bay. Since mussels are a major part of this bird's diet, we cannot ignore the possibility of mussel poisoning as being the cause of death.

The Maine coast bordering on New Brunswick has given us the greatest concern because of the Canadian experience of high toxicity in that area. Further the Canadian observations that conditions for high toxicity are worse when the mean annual water temperatures are the lowest and their hydrographers prediction of a long range decline, made it imperative that an assessment of paralytic shellfish poisoning be made on our coast.

Therefore, early in July with the assistance of Mr. Earl McFarren of the U.S.P.H.S., Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio a testing program was started.

The bioassay procedure described in "Interim Plan for the Standardization of Paralytic Shellfish Poison by Use of a Reference Standard" was used.

Jackson Memorial Laboratory black mice, strain BAF were used in the bioassay. The C.F. value for this strain was .31 which meant the sensitivity of the test was 62 ug. per 100 grams meat.

Since we had no information concerning the presence of shellfish toxin, the purpose of the survey was first to see if the toxin was present in the shellfish and second, if the toxin was present, to determine the area involved, and third to select monitoring stations for control purposes.

The first sampling was processed July 9 and showed measurable amounts of poison in clams collected from the Lubec channel area July 1.

A second and more intensive sampling of the Eastport, Lubec and Cobscook Bay was undertaken on July 10. The results of this sampling showed a peak of 204 ug. in clams and 656 ug. in

mussels in the Lubec - Quoddy area with borderline amounts in the Eastport and approaches to Cobscook Bay areas.

The area from Lubec to West Quoddy was closed to the taking of shellfish.

Unfortunately no samples were taken in the border area from the 10th to 22nd of July. During this period we shifted laboratories from Boothbay Harbor to a mobile laboratory nearer the affected area.

The samplings taken after July 22 showed a marked decrease in the poison content to a point in the first week of August when the poison content was beyond the sensitivity of our test.

The farthest westward we found traces of poison was at Bailey's Mistake and none was found in the Hill Cove and Lewis Cove areas of the St. Croix River.

Samples of mussels and clams were taken at the points of land as far west as Boothbay Harbor, none showed any measurable toxin.

Hydrography

One can only speculate about the relation of the hydrography to the level of toxicity in Eastport and West Quoddy since detailed data is not available.

The tidal range and topography are probably the two most important factors which influence the hydrography of this locality. The mean tidal range at Eastport is 18.2; Lubec, 17.5; West Quoddy Head, 15.7; and Cutler, 13.9. From a quick glance at the map, it is evident that outside waters enter Passamaquoddy Bay through very narrow passages. The waters in the vicinity of these passages are obviously turbulent and presumably well mixed.

The generalization apparently holds true, as in other areas studied, that the nearer the open water the likelihood of higher levels of toxicity is greater.

Shellfish from the immediate vicinity of West Quoddy Head were generally higher in toxicity than those from farther in the embayment. These were probably more heavily affected by the water from the southeastern shore of Campobello Island than from the waters entering the Deer Isle - Campobello Island passage.

It is assumed, because of the level of toxicity at Station 3 and 17 that on the ebbing tide, well mixed waters leave the Lubec channel with a relatively great speed, holding in a field until well out in the Grand Manan Channel before dispersion takes place.

References

U. S. Fish and Wildlife Service (1946) A Report of a Conference on Mussel Poisoning, August 6, 1946.

Scattergood (1958) Personal correspondence.

TABLE I

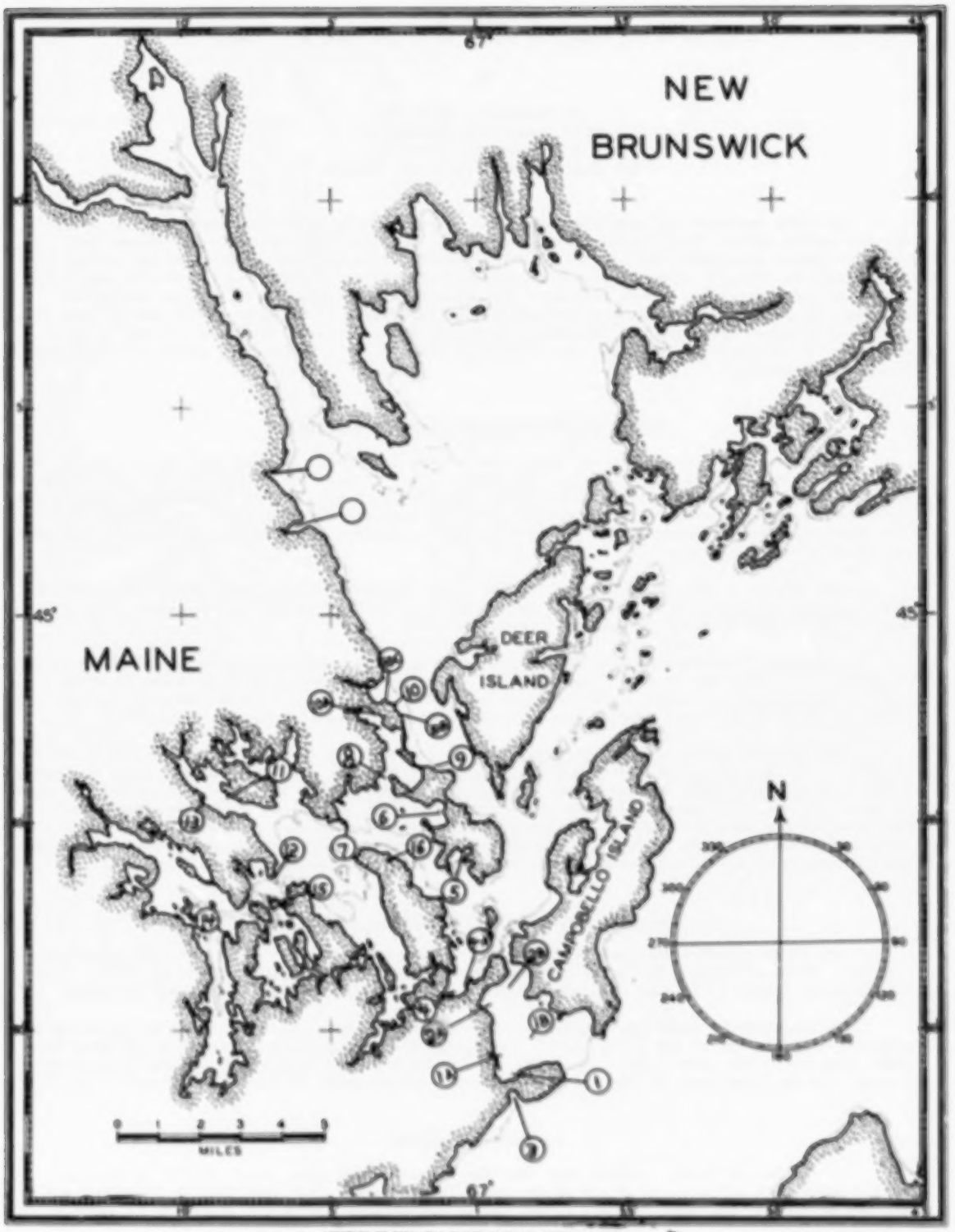
Paralytic Shellfish Bioassay

Date	Location	Station	Shellfish	u/g / 100 gm	Remarks
7- 1-58	Lubec Channel	1	Clams	69.1	
7-10-58			"	152	
7-10-58	Lubec Channel	1A	Clams	130	
7-24-58			"	94	East of Bar
7-24-58			"	109	West of Bar
7-29-58			"	79	
7-10-58	Lubec Channel	1B	Clams	204	East of Bar
7-24-58			"	104	West of Bar
7-24-58			"	91	East of Bar
7-29-58			"	160	
8- 5-58			"	56	
8-13-58			"	<62	
7- 1-58	Lubec Channel	2A	Clams	65	
7-10-58			"	198	
7-29-58			"	<62	
7-10-58	Lubec Channel	2B	Clams	82	
7-10-58			Mussels	656	
7-29-58			"	73	
6-25-58	S. Lubec, Carrying Place C.	3	Clams	<62	
7-24-58			"	74	
8- 5-58			"	<62	
8-13-58			"	<62	
7- 1-58	Johnson Bay	4	Clams	<62	
7-10-58			"	<62	
7-23-58	Diamond Head	4A	Clams	<62	
7-10-58	Broad Cove - Eastport	5	"	<62	
7-10-58	Carrying Place Cove, Eastport	6	Clams	66	
7-22-58			"	<62	
7-10-58	Birch Point	7	Clams	75.5	
7-22-58			"	<62	Inshore
7-22-58			"	<62	Offshore
7-10-58	Old Ferry Bridge, Eastport	8	Clams	68.7	
7-22-58			"	<62	MDT = 19:11
7-10-58	Moose Island, Eastport	9	Clams	85	
7-22-58			"	77	Inshore
7-22-58			"	74	Offshore
8- 5-58			"	<62	
8-13-58			"	<62	
7-10-58	Frost Island, Eastport	10	Clams	71	
7-28-58			"	61	
8- 5-58			"	<62	
8-13-58			"	<62	
7-22-58	Bar Harbor, Eastport	10A	Clams	<62	
7-22-58	Frost Island, Eastport	10B	Clams	110	
7-22-58			Periwinkles	<62	
7-28-58			Clams	71	
7-28-58	Frost Island, Eastport	10C	Clams	<62	
7-22-58	Pennamaquan River		"	<62	
7-23-58	Leighton's Pt.	12	Clams	<62	MDT = 16:07
7-23-58	Leighton's Pt.	13	"	<62	
7-23-58	Trescott	14	"	<62	
7-23-58	Denbons Pt., Young's C.	15	"	<62	
7-25-58	Cove Pt., N. Lubec	16	"	63	
7-24-58	Bailey's Mistake	17	Clams	<62	MDT = 24:02
7-29-58			"	<62	
7-10-58	Lewis Cove, St. Croix River		"	<62	
7-10-58	Mill Cove, St. Croix River		"	<62	
7-29-58			"	<62	
7-29-58			"	<62	

TABLE II

Paralytic Shellfish Bioassay

Date	Location	Shellfish	u/g / 100 gm	Remarks
7- 2-58	Little Machias Bay, Cutler	Clams	<62	
7-19-58	" " " "	Mussels	<62	
7-19-58	" " " "	Clams	<62	
7-29-58	" " " "	"	<62	
7- 2-58	Holmes Bay	"	<62	
7- 6-58	Machiasport, Randall Pt.	"	<62	
7-19-58	" " "	"	<62	
7- 7-58	Roque Bluffs, Pond Cove	"	<62	
7-19-58	" " " "	"	<62	
7-19-58	" " " "	Mussels	<62	
7-24-58	Rodger's Island Bar, Jonesport	Clams	<62	
7-24-58	Beals Is. Alley Bay, Jonesport	"	<62	Eastside
7-24-58	" " " " "	"	<62	Westside
7-28-58	Schoodic Head, East Pond	"	<62	
7-28-58	Schoodic Head, West Pond	"	<62	
7-28-58	Hancock Point	"	<62	
7- 7-58	Mt. Desert Is., Salisbury Cove	Mussels	<62	
7-28-58	Mt. Desert Is. Clarks C., S. W. Harbor	Clams	<62	
7-28-58	Mt. Desert Island, S. W. Harbor	"	62	
7-27-58	South Bluehill	Mussels	62	
7-27-58	Bluehill Bay	"	62	
7-27-58	Naskeag Pt.	"	62	
7-27-58	Flye Pt., Brooklin	Clams	62	
7-22-58	Castine	Mussels	62	
7-22-58	Searsport	"	62	
7-22-58	"	Clams	62	
7-22-58	Lincolnville Beach	Mussels	62	
7-19-58	Boothbay Harbor	Clams	62	



LOCATION OF PUBLIC FISH SAMPLING STATIONS IN MAINE

APPENDIX H

A REVIEW OF THE PARALYTIC SHELLFISH POISON PROBLEM IN CANADA

by

Mr. Ronald H. Bond, OIC
Fish Inspection Laboratory
Department of Fisheries
St. Andrews, New Brunswick, Canada

Periodic outbreaks of paralytic shellfish poisoning have occurred in Canada since 1793.(1) but the public health significance of the problem was not fully appreciated until Sommer and Meyer's studies were published in 1937.(2) Their publication, following two fatal poisonings in Nova Scotia in 1936,(3) prompted investigations on both the Atlantic(4) and Pacific(5) coasts to determine the seriousness of the problem. Our present Bay of Fundy classification system has evolved from a co-operative study undertaken by the Department of National Health and Welfare, the Fisheries Research Board and the Department of Fisheries in 1943.(6) A similar scheme is now operating in the Province of Quebec but efforts to classify British Columbia shellfish areas have been less successful.

Source of the Poison and Species Affected

Plankton studies in the Bay of Fundy area of New Brunswick indicate that the origin of the poison is the dinoflagellate, Gonyaulax tamarensis.(7,9) It has been shown that shellfish become toxic as soon as Gonyaulax appear in the water, that peaks in the numbers of Gonyaulax present are reflected by peaks in the poison content of shellfish and that toxicity begins to decline when this dinoflagellate disappears from the surface water. No such relationship could be demonstrated for other dinoflagellates.

The same or a similar species is thought to be the cause of poisonous shellfish in Quebec, and Gonyaulax catenella is considered to be the responsible agent in British Columbia although there is no direct evidence to support these views.

Presuming dinoflagellates to be the source of poison in shellfish, then any plankton feeder is potentially dangerous in known toxic waters. This fact was demonstrated by exposing eastern oysters and quahaugs to water where shellfish were known to be toxic. (These species are not normally found in our toxic areas). In less than a week both species became toxic.(8)

The horse mussel(9,10) (Volsella modiolus), blue mussel(1,3) (Mytilus edulis), soft-shell clam(9,11,12) (Mya arenaria), bar clam(12) (Spisula solidissima), butter clam (Saxidomus giganteus), oyster(15) (Ostrea gigas) and scallop(9) (Placopecten magellanicus) have been responsible for cases of paralytic shellfish poisoning. The scallop deserves special mention -- the adductor mussel is the only portion normally eaten and it has never been found to be toxic. However, the "roes" are occasionally eaten and at least one human poisoning has been attributed to them. There have been many instances of animal poisoning by scallop "rims" and other species of shellfish.(9,12)

Incidence and Distribution of Toxic Shellfish

The first recorded outbreak of poisoning occurred on Vancouver Island, British Columbia, in 1793.(1) Three of Captain Vancouver's seamen were ill after eating mussels, and one died. Also on Vancouver Island in 1942 an outbreak occurred, and in 1957 there were cases, but no deaths.

On the Atlantic coast there are two widely separated areas in which shellfish poisoning has occurred. In the Bay of Fundy regions of Nova Scotia and New Brunswick, a total of 60 cases have been reported in the years 1889, 1934, 1936, 1945, 1957 and 1958.(3,9,10,12) In the Gaspe area of Quebec, two children died in 1948 and seven persons were poisoned in 1954 - two of whom died.(11)

Seasonal Variations

In the Bay of Fundy, records over the last sixteen years show that toxicity follows a fairly consistent pattern. High scores can occur in most areas from June through September and for the balance of the year they are either low or non-existent, although shellfish in certain

areas have been known to retain toxicity throughout most of the year. Toxicities in Quebec show a similar seasonal variation.

No clear pattern has been established on the west coast. A possible explanation lies in the anatomical distribution of the poison. In Pacific butter clams, the poison is concentrated in the siphon where it can remain for long periods after the causative organism has disappeared from the water.(5) Atlantic shellfish invariably accumulate the greatest load in the digestive gland and gills from where it can disappear rather quickly when the poisonous plankton are no longer present in the food supply.(9)

In the Bay of Fundy, appearance of Gonyaulax tamarensis has been directly related to a summer threshold water temperature.(7) The amount of toxicity in shellfish has been demonstrated to vary inversely with the winter water temperature.(13) These same winter temperatures are the determining factors for when the threshold temperature will be reached in any given year.

Control of Toxic Areas

There is a better understanding of the problem in the Bay of Fundy than elsewhere in Canada and, through long experience, a workable control scheme has been developed, based on area classification and the consistent appearance of poison at Campobello Island about ten days before it is found in mainland areas. The basic scheme was first used in 1948(14) and has been revised from time to time to keep pace with expanding knowledge of the problem. At the present time, shellfish grounds fall into either of two classifications: those that may be toxic for short periods each year (usually 2 to 3 months) and those that are potentially dangerous the year round. A 400 mouse unit score at our Campobello sampling station is used as the signal score for closure of all New Brunswick areas, and all individual areas must score consistently below 400 m.u. before they are re-opened to the shucking trade. Canning is permitted when scores range below 1000 m.u. but all packs must be tested and certified poison free before they are released for sale.

The Quebec control scheme parallels the one just described and has shown satisfactory results over the past four years. At the present time, the scheme includes all areas on the south shore of the St. Lawrence River and in the Bay of Chaleur as well as a section of the north shore of the River. Any new areas receive a sanitary survey and are tested for the presence of poison before exploitation is permitted.

Very little has been published on the problem in British Columbia. Best available information indicates that known toxic areas are sampled and closed when poison is demonstrated to be present.(15)

It must be emphasized that although industrial control has been adequate on the east coast, we have not been successful in protecting picnickers and other casual diggers.(9,11,12) Of the 79 recorded poisonings (Table I), 69 have occurred since the inception of control schemes and all but two have been among picnickers or local residents who dug their own clams. These poisonings have occurred in spite of prominently posted warning notices and point to the need for a concerted effort to increase public appreciation of the problem. The use of newspaper, television and radio announcements in the past several years has been a step in this direction.(12)

Assay Methods and Standards

All east coast shellfish extracts are assayed by the Laboratory of Hygiene in Ottawa using the standard bioassay procedure (CF value 0.16). The use of raw shellfish containing up to 400 m.u. (64 u.g.) is permitted and clams which have been canned must be certified poison free.

There are some who consider the 400 unit standard too low and it is true that the lowest scoring clams which have caused poisoning were in excess of 6,000 m.u. per 100 g.(9,11) However, we know that adults have suffered severe poisoning from a 5,500 m.u. dosage and have experienced mild symptoms from dosages of 1,900 and 2,150 m.u.; a two-year-old child was seriously ill after a dosage of only 600 m.u.(12) These data have discouraged any thought of increasing the 400-unit standard approved by the Canadian Department of National Health and Welfare and the United States Department of Health, Education and Welfare.

TABLE I

REPORTED CASES OF PARALYTIC SHELLFISH POISONING

Date	Location	Species Eaten	No. of Cases	Deaths
1793	British Columbia	Mytilus edulis	3	1
1889	New Brunswick	Volsella Modiolus	1	1
1934	Nova Scotia	Placopecten magellanicus	1	-
1936	Nova Scotia and New Brunswick	Mytilus edulis and Volsella modiolus	6	2
1942	British Columbia			
1945	Nova Scotia and New Brunswick	Mya arenaria	26	-
1948	Quebec	Mytilus edulis	2	2
1954	Quebec	Mya arenaria	7	2
1957	New Brunswick	Mya arenaria and Spisula solidissima	33	-
	British Columbia	Ostrea gigas	ca 50	-
1958	New Brunswick	Mya arenaria	1	-

REFERENCES

1. Vancouver, Capt. George, A Voyage of Discovery to the North Pacific Ocean and Around the World, 2, 285, 1798.
2. Sommer, H. & K. F. Meyer. Arch. Path. 24(5), 560-598, 1937.
3. Murphy, A. L. Canad. Med. Assoc. J., 35, 418-419, 1936.
4. Gibbard, J. et al. Canad. Pub. Health J., 30, 193-197, 1939.
5. Pugsley, L. I. Fish. Res. Bd. Can., Prog. Rept. Pac., 40, 11-13, 1939.
6. Medcof, J. C., Toxicity Records 1943 (unpublished), Fish. Res. Bd. Can. MS. Rep.
7. Needler, A. B., J. Fish. Res. Bd. Can., 7(8), 1949.
8. Bond, R. M. Toxicity Records, 1957 (unpublished), MS. Rep., Canad. Dept. Fish.
9. Medcof, J. C. et al, Fish. Res. Bd. Can., Bulletin 75, 1947.
10. Ganong, W. F. Bull. Nat. Hist. Soc. N. B. 8, 1-116, 1889.
11. Tennant, A. D. et al. Canad. Med. Assoc. J., 72, 436-39, 1955.
12. Bond, R. M. & J. C. Medcof Canad. Med. Assoc. J. 79, 19-24, 1958.
13. Bond, R. M. Toxicity Records 1956, MS. Rep. Canad. Dept. Fish. (unpublished)
14. Medcof, J. C. Toxicity Records 1948, MS. Rep. Fish. Res. Bd. Can. (unpublished)
15. Davies, F. R. E. et al. Canad. J. Pub. Health, July, 1958.

APPENDIX I

WASHINGTON STATE PARALYTIC SHELLFISH POISON SURVEILLANCE

by

John G. Girard
Shellfish Sanitarian
Washington State Department of Health
Seattle, Washington

The Paralytic Shellfish Poison Surveillance Program was reactivated in the spring of 1957. The earlier program which was initiated in 1932, had been terminated in 1946. From this early work it was determined that the most critical shellfish areas during certain periods were the ocean beaches and the Straits of Juan de Fuca eastwardly to Dungeness Spit. By regulation both the State Department of Health and the State Department of Fisheries close these areas for the harvesting of shellfish other than razor clams (Siliqua patula) between April 1, and October 31, of each year. Razor clams were excluded from the regulations as the toxin is evidently concentrated in the digestive system, which is not consumed by man.

In May of 1942 some of the shellfish suffered an increase in toxicity, with the shellfish in the Straits of Juan de Fuca and the ocean beaches reaching extremely high levels of toxin. It was at this time that there were two deaths from eating mussels (Mytilus californianus) and one from eating butter clams (Saxidomus giganteus) collected from the Straits of Juan de Fuca. It was also at this time that the pacific oyster (Crassostrea gigas) was found to be able to concentrate the shellfish toxin.

The control program is made extremely difficult due to a number of reasons. The 2600 miles of marine shorelines, with many ecological variations, certainly is of importance. The rather large number of species of shellfish and their evident ability to concentrate the toxin at different rates also creates difficulties. Last but not least is the ability of at least one species (Saxidomus giganteus) to retain the toxin for very long periods of time. This last item is of particular importance, as this species grows in the same area as the native little neck (Protothaca staminea) which is either poor in concentrating the toxin or gives it up at a very rapid rate.

A limited surveillance program was accomplished during the spring and summer of 1957. On being advised of the prevalence of toxin in British Columbia shellfish in the fall, our sampling program was increased to include all species of commercial shellfish and from all commercial areas most likely to be affected. The findings indicated that the toxin was in concentrations above the permissible limits in two different species of shellfish in two separate areas.

In the Stackpole area of Willapa Harbor, pacific oysters were found to have concentrated toxin in levels up to 2200 M.U. per 100 gram of meat. The area was closed for the harvesting of shellfish for the fresh market. Based on one series of samples, testings of shell stock and the heat sterilized product from a canner using oysters from this area indicated that approximately a 75% reduction of toxin occurred in the conventional heat sterilization of pacific oysters.

The other area of concern was the Washington Harbor - Discovery Bay area. This area lies immediately east of Dungeness Spit, which is the eastern limits of the seasonal closure line of the Straits of Juan de Fuca. The toxin was found to exceed slightly the permissible limits in the butter clam. Harvestings of this species were directed to the canners for the heat sterilized pack.

The present testing program attempts to satisfy the needs of all commercial shellfish areas as well as some sports areas. Samples of shellfish are submitted bi-monthly for toxicity determination from areas where the toxin is most likely to occur. At this time no attempt is being made to identify the causative organism, nor to discover under which conditions it may be expected to occur. Certainly this information is necessary if a control program is to succeed.

APPENDIX J

THE SIGNIFICANCE OF PINK DISCOLORATION IN SHELLFISH

by

Mr. Donald W. Lear
Chesapeake Biological Laboratory
Maryland Department of Research and Education
Solomons, Maryland

Commercial molluscan shellfish packs can certainly be colorful things. The literature describes many colors of commercial shellfish: green, blue-green, yellow, pinks, and red. Today, however, we will limit ourselves to discussion of the pinks and reds.

There are four causes of red or pink coloration described in the literature:

1. Pink yeast
2. Red coloration ascribed to ingestion of the microscopic plant Gymnodinium splendens
3. "Sponge" oyster crabs, Pinnotheres, in the pack, and
4. Another plant pigment, as yet unidentified.

The first mentioned of these, pink yeast, is due to the presence in the pack of a Torula-type yeast. The work of Puncochar, et als., in 1941, indicates all oysters, from New England to the Gulf of Mexico, contain pink yeasts. The control of these microorganisms is through proper plant sanitation and proper handling.

Another cause of red coloration was reported by Dr. Thurlow Nelson, in an outbreak of red oysters in Delaware Bay. In this case, the red was associated with a phytoplankton "bloom" or "red tide," of Gymnodinium splendens, in the waters over the oyster beds. This species, G. splendens, is not toxic, but another species of the same genus, G. brevis, has been involved in the toxic Florida red tides.

Still a third cause of red coloration in oysters has been attributed to the packing of the little oyster crabs, Pinnotheres, when the females are in sponge condition, that is, when carrying eggs. Apparently the color diffuses from the eggs into the pack, producing a red coloration.

Now a fourth cause, similar in some respects, but apparently differing in others, has come to light. In this case, red coloration appeared in the pack of soft clams, Mya arenaria, from the Chesapeake. Under pretty much standardized conditions of shucking houses, the pack of soft clams in the cool and cold months sometimes develops a very definite beet-juice or blood red coloration in the liquor after 3 to 5 days storage under proper refrigeration. The predisposition for color development is usually not evident at the time of shucking.

This past year the Chesapeake Biological Laboratory at Solomons, the Chesapeake Bay Institute of the Johns Hopkins University in Baltimore, and the Fish and Wildlife Service Shellfish Laboratory at Annapolis combined efforts to investigate the matter. Consultation from other laboratories on the East Coast was utilized also.

There seem to be three major differences between this phenomenon and that reported as a Gymnodinium splendens outbreak by Dr. Nelson. First, this phenomenon occurred in soft clams, not in oysters. Apparently oysters from this same geographical region gave no trouble. Secondly, no red "tide," as such, was evident. Third, the duration of red coloration in soft clams apparently differs from oysters. In the case of oysters, red coloration outbreaks are generally of two to four weeks duration, and then oysters harvested from the same waters are "normal" again. In the case of soft clams, however, in the Chesapeake in the region of the Bay Bridge, the initial appearance of red coloration was reported the first week in October, 1957, and persisted throughout the entire winter, as it has been reported in some years past. This did have advantages from the biologist's standpoint, for it offered a steady supply of fresh material to study.

In the course of the past winter we found out several things about this phenomenon. We think the development of red coloration is not a growth phenomenon, because color developed in the presence of substances inimical or inhibitory to the growth of organisms. Color developed, in some cases faster, in these than in the controls. We used bichloride of mercury, lysol,

penicillin, streptomycin, and silver nitrate. In all cases color developed. We did find, however, that color would not develop in formalin or forty percent formaldehyde solution.

The locus of color production in the clam seems to be the small brown "liver" or digestive gland. This was indicated by dissecting various parts of potentially red clams, and color was released only from the "liver."

The red color, or pigment, is stored as a red pigment, not as a non-red pigment that later converts to a red color. This was indicated by streak "chromatograms," in which a small portion of the "liver" was streaked on filter paper, and a red "front" could be observed moving out with the moisture. Phil Nelson, at U. S. Fish and Wildlife Shellfish Laboratory at Annapolis, was able to grind and filter the "liver," and recover red coloration in the filtrate.

The rate of development of red coloration, under refrigeration, is influenced by the salt concentration of the surrounding medium.

Heating at 50°C. for 15 minutes modifies or destroys the pigment; 40°C. for 30 minutes did not alter pigmentation.

Clams held as shellstock for 10 days did not develop red coloration, while shucked clams from the same lot did develop red coloration.

Samples of red and non-red clams sent to the U. S. Public Health Service Laboratory in Cincinnati for assay for shellfish toxins were reported negative.

The color varied somewhat, from a beet-juice red, even the color of maraschino cherries, to an orange brown. Extracting in organic solvents invariably yielded an amber or yellow color, with no red evident.

Bacteriological plating yielded no red chromogenic bacteria at Chesapeake Biological Laboratory.

Dr. Carritt, at the Chesapeake Bay Institute, tentatively identified the pigment as a plant carotenoid, by extraction, adsorption and spectrophotometric techniques.

Phil Nelson, at the Fish and Wildlife Shellfish Laboratory at Annapolis, has accumulated evidence which strongly indicates red pigment is stored in the "liver" of the clam, and in the cold months, the clam is not able to metabolize rapidly enough to rid itself of the red pigment. Holding these clams at higher temperatures, about 20°C., enables the clam to clear itself in a few days.

It is still unknown whether the ingested phytoplankter, which is the source of red pigment, is a warm water or cold water species or even several species.

We do not know whether any single causative agent can produce red coloration in both soft clams and oysters in the Chesapeake.

Some thought has been given to a practical system for predicting whether or not a given clam will turn red. This is especially significant because of the fact that the pack generally requires 3 or more days before color appears. All of the following have been considered, but not developed to the stage where they could be used in industry:

1. The filter paper streak chromatograms already mentioned were tried, and found to be about 67 percent accurate under the conditions we tested them.

2. The filtration technique attempted by Phil Nelson might be developed.

3. Dr. Carritt discovered an unusual fluorescence of red clam material under ultraviolet lighting, which might be developed as a predicting tool.

What then does this fourth cause of red coloration seem to be? It would seem to be related to Dr. Nelson's Gymnodinium blooms, except in this case no coincident phytoplankton blooms have been observed, despite regular sampling. This red coloration in the clams, persisting throughout the winter, would seem to be due to the ingestion of the microflora of the water in the colder months, but not from a population of "red tide" proportions. In fact, the water in the bay in the Annapolis - Kent Island region was quite clear last winter.

What is the extent of the economic importance of "red coloration" to the shellfish industry? Dave Wallace, in a meeting at Annapolis last December, estimated losses due to red coloration at $100,000 to $250,000 per year in rejections alone.

What should be the direction of future research? I have heard several suggestions discussed informally which might prove profitable:

1. A study of the pigment itself, to determine its properties and to find a method for altering it physically or chemically in the pack. This seems to be the most direct attack from a practical angle.

2. The identification and study of the causative organism in the water would lead to a prediction of potential red coloration, and possibly to control measures somewhere in the chain of production between the environment of the clam and the consumer.

3. A third problem should be followed. It has not been established whether or not red coloration in soft clams and in oysters in the Chesapeake are due to the same cause. This could probably be quite readily worked out, using known techniques.

APPENDIX K

ATTENDANCE AT 1958 SHELLFISH SANITATION WORKSHOP

ATKINS, C. H.
Public Health Service

BAUER, Theodore J.
Public Health Service

BELLEVILLE, Laurier
Dept. of National Health and Welfare, Canada

BENDER, Maurice
U. S. Fish & Wildlife Service

BENNETT, Quentin R.
N. Y. State Dept. of Conservation

BERRY, Roscoe W.
Virginia State Dept. of Health

BOND, Ronald M.
Canadian Dept. of Fisheries

BOWERS, J. Paul
Public Health Service

BRINSFIELD, Carroll S.
Maryland State Dept. of Health

BROWN, Robert M.
Maryland State Dept. of Health

BROWNLEE, Wesley J.
Canadian Dept. of Fisheries

BYRD, G. Clifford
National Fisheries Institute

CALDWELL, Neil McKeithen
N. C. State Board of Health

CARLEY, George
Public Health Service

CARY, William H., Jr.
D. C. Dept. of Public Health

CEURVELS, A. Russell
Mass. Div. of Marine Fisheries

CHIPMAN, Walter A.
U. S. Fish & Wildlife Service

CULLEN, Douglas
Dept. of National Health and Welfare

D'AGUANNO, William
Food and Drug Administration

ENGLE, James B.
U. S. Fish & Wildlife Service

FAULKNER, John D.
Public Health Service

FLETCHER, Cecil C.
Public Health Service

FORTGANG, Albert
N. Y. City Dept of Health

FOWLER, Sam
Maryland State Dept. of Health

FRITZ, John H.
D. C. Dept. of Public Health

GIRARD, John
Washington State Dept. of Health

GOGGINS, Phillip L.
Maine Sea & Shore Fisheries

GREGORY, Robert H.
Virginia State Dept. of Health

GREY, Shelbey
Food and Drug Administration

GROSSMAN, R. Frank
Public Health Service

HARRIS, Eugene
Public Health Service

HAVEN, Dexter
Virginia Fisheries Laboratory

HERZIK, G. R., Jr.
Texas State Dept. of Health

HEYDECKER, Wayne D.
Atlantic States Marine Fisheries Commission

HIERS, Hart B.
S. C. State Health Dept.

HOBBS, Lewis F.
Maryland State Dept. of Health

HOLY, William E.
Public Health Service

HOPE, John
Virginia State Dept. of Health

HOUSER, Leroy S.
Public Health Service

JACKSON, E. Russell
Florida State Board of Health

JEFFRIES, Norman F.
Greenwich Oyster Co.

JENSEN, Eugene T.
Public Health Service

JOHNSON, Fred
Bay Food Products Co., Inc.

KELLY, C. B.
Public Health Service

LANCASTER, Harry R.
Department of the Army

LEAR, Donald W.
Maryland Dept. of Research and Education

LEE, Charles F.
U. S. Fish & Wildlife Service

LENERT, Louva G.
Georgia State Dept. of Public Health

LEWIS, Keith H.
Public Health Service

LIEBERMAN, Joseph
Atomic Energy Commission

LUPTON, Howard
N. C. State Board of Health

MACOMBER, Ronald G.
Public Health Service

MARK, Richard S.
Public Health Service

McCORMACK, Rudolph
S. C. State Board of Health

McFARLAND, Bill V.
Food and Drug Administration

McGINNES, T. D.
Virginia Seafoods, Inc.

MENZIES, J. Ross
Dept. of National Health and Welfare, Canada

MEREDITH, E. C.
Virginia State Dept. of Health

MEYERS, H. F.
Public Health Service

MILES, William F.
J. H. Miles and Co., Inc.

MORGAN, Cranston
W. F. Morgan & Sons

MULLAN, H. Wendell
Canadian Dept. of Fisheries

NOVAK, Arthur F.
Louisiana State University

PEARSON, James C.
Food and Drug Administration

PELLAN, Wilfrid
Provincial Government of
Health, Quebec

PERRY, C. A.
Maryland State Dept. of Health

PIERCE, George O.
D. C. Dept. of Public Health

POWELL, John J.
Department of the Army

PRINGLE, Somers
S. C. State Board of Health

QUAYLE, Daniel B.
Pacific Biological Station
Canada

RAWLINSON, R. K.
The Vollrath Co.

ROY, Jean
Department of Health
Quebec

RUPPERT, Edwin L.
Public Health Service

RUSHMORE, R. W.
Department of the Air Force

SALINGER, Arnold
Maryland State Dept. of Health

SAVAGE, Lee
Savage & Mears

SHAMHOLTZ, Mark I.
Virginia State Dept. of Health

SCHANTZ, Edward J.
Army Chemical Corps

SCHMIDT, Gail D.
Public Health Service

SHERMAN, Leslie K.
Conn. State Dept. of Health

SILVA, Frank J.
Delaware State Board of Health

SLOCUM, Glenn G.
Food and Drug Administration

TAYLOR, Worthen H.
Mass. Dept. of Public Health

TENNANT, Alan D.
Dept. of National Health and
Welfare, Canada

THATCHER, Fred
Food and Drugs Directorate
Canada

TIMKO, Francis A.
N. J. State Dept. of Health

UDELL, Harold
N. Y. State Conservation Dept.

VALEAR, Paul E.
D. C. Dept. of Public Health

VANDERWERKER, Ralph
Public Health Service

WALLACE, David H.
Oyster Institute of North America

WATSON, Ralph E.
Ralph E. Watson Oyster Co.

WILEY, Cloyde W.
Virginia State Dept. of Health

WOODFIELD, William R.
Woodfield Fish & Oyster Co.

GPO 873890